AGENCY FOR INTERNATIONAL DEVELOPMENT
BUREAU FOR AFRICA
Office of Technical Resources
AFR/TR/ANR
Washington, DC 20523-1515

September 18, 1991

MEMORANDUM

TO: ADO's of Sahelian USAID's

FROM: Walter I. Knausenberger, AFR/TR/ANR/NR

SUBJECT: Report on a neem tree disease in Niger

Upon the request of USAID/Niger and Care International, and to follow-up on our related telegram of o/a 23 September, 1991, we are pleased to send you the enclosed copy of a report by a consultant plant pathologist sent to Niger to investigate a suspected neem disease. This consultant was sent to Niger under the Forestry Support Program through an AFR/TR/ANR/NR buy-in to S&T/FENR.

The implications of this disease, which potentially could lead to the decline of this "cornucopia tree" over large areas, are significant for the future of agroforestry in the Sahel. However, if the disease is indeed confirmed to be fungal in nature, then the steps to intervention and prevention of spread are rather straightforward in principle. The appearance of this disease should, however, also reinforce the conviction that monoculture of any species is to be avoided.

A follow-up investigation is planned in Niger for October/November 1991. Any new information will be shared as soon as possible.

cc:
AFR/TR/ANR/NR, J.Gaudet
AFR/TR/ANR/NR, M.McGahuey
AFR/TR/ANR, B.Stoner

AFR/TR/ANR/NR:WKnausenberger:ww:neem1.5

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AFR/TR/ANR/NR:JGAUDET {SUBST} AFR/TR/ANR/NR:WTHOMAS {DRAFT}
AFR/TR/ANR/NR:MMCGAHUEY {INFO} AFR/SWA:JROYER {DRAFT}
USDA/FS/FSP:JMORRIS {INFO} USDA/OICD/NRE:BCROSSAN {INFO}

ROUTINE NIAMEY

ROUTINE ABIDJAN, BAMAKO, BANJUL, BISSAU, DAKAR, NDJAMENA, NOUAKCHOTT, OUAGADOUGOU, PRAIA, YAOUNDE

AIDAC FOR ADO'S, NIAMEY FOR G. TAYLOR AND B. RAND;

E.O. 12356: N/A

TAGS:

SUBJECT: NEEM TREE DISORDER IN NIGER: FOLLOW-UP OPTIONS

ABIDJAN FOR ADO, REO, PASS TO USDA-APHIS;

REF.: {A} NIAMEY 5867 {NOTAL}

SUMMARY: THE EXISTENCE OF AN APPARENTLY WIDELY DISPERSED DISEASE AFFECTING LARGE NUMBERS OF NEEM TREES IN NIGER HAS BEEN CONFIRMED. THE DISEASE CAUSES A DECLINE PROVISIONALLY DIAGNOSED AS AN INFECTION BY A SOIL-BORNE FUNGUS. PERMANENT WILT, DENUDED, OFTEN GUMMY SHOOTS AND TRUNKS, BRANCH DIE-BACK, AND TREE DEATH WITHIN A YEAR OF FIRST SIGNS, ARE AMONG THE SYMPTOMS. THIS DISORDER HAS THE POTENTIAL OF BEING A DETERMINANT IN THE SUCCESS OR FAILURE OF NEEM PLANTING PROGRAMS IN THE SAHEL. GIVEN THE ECONOMIC AND BIOLOGICAL SIGNIFICANCE OF THIS TREE, CONFIRMATORY INVESTIGATIONS ARE STRONGLY RECOMMENDED. OPTIONS FOR FOLLOW-UP BY USAID/NIGER ARE REVIEWED. OTHER SAHELIAN MISSIONS ARE URGED TO BE ON THE LOOKOUT FOR THIS DISEASE OF NEEM TREES. THE CONSULTANT'S REPORT IS BEING POUCHED TO ALL SAHELIAN MISSIONS. ACTIONS REQUESTED OF USAID/NIGER ARE GIVEN IN PARA. 8. END SUMMARY.

1. FOLLOWING THE TWO-WEEK TECHNICAL ASSISTANCE MISSION IN JUNE/JULY 1991 BY PLANT PATHOLOGIST DR. G.K. BATRA, TO INVESTIGATE THE NATURE

OF A DISORDER OF NEEM TREES IN NIGER, SEVERAL REPORTS HAVE BEEN PRODUCED BY DR. BATRA. THESE INCLUDE THE FIELD INVESTIGATION REPORT AND SUBSEQUENT LABORATORY ANALYSIS REPORTS, THE LATTER PROVIDED THROUGH AN ADDITION TO DR. BATRA'S BUDGET UNDER THE AFR/TR/ANR FSP BUY-IN TO S&T/FENR. THE REPORTS CONFIRM BATRA'S DEBRIEFING (REFTEL) THAT AN INFECTIOUS DISEASE IS INVOLVED. ELEVEN SITES IN THE NIAMEY-TAHOUA-MARADI SECTOR HAD AFFECTED TREES. DISEASE INCIDENCE OBSERVED VARIED FROM UNDER 10 PERCENT OF TREES IN A MATURE PLANTATION TO OVER 75 PERCENT IN SOME YOUNGER PLANTATIONS. MOUNTING A COMPREHENSIVE SURVEY OF ARRONDISSEMENTS HAVING CONCENTRATIONS OF NEEM TREES WOULD SEEM JUSTIFIED.

2. A. TO ASSIST OTHERS IN RECOGNIZING THE PROBLEM, THE MAIN SYMPTOMS OD THE DECLINE ARE REVIEWED HERE:
- YOUNG LEAVES TURN DULL GREEN OR PALE YELLOW;
- LEAVES OFTEN DEFORMED, CRINKLED, FOLDED-IN AND COMPRESSED NEAR BRANCH TIPS, GIVING BUNCHY APPEARANCE;
- BRANCH TIPS ARE STUNTED, WITH SHORTENED DISTANCES BETWEEN NODES AND LEAF SCARS;
- BARE TIPS OFTEN ARE PERMANENTLY WILTED, YELLOWED, EXUDING A SHINY, CLEAR WHITE TO TRANSLUCENTLY YELLOW GUM;
- BRANCHES DIE BACK FORM THE TIP, LEAVING BEHIND BARE, DEAD, SCORCHED BRANCHES, EVENTUALLY LEADING TO DEATH OF THE ENTIRE TREE (WITHIN SEVERAL MONTHS TO A YEAR);
- TREES USUALLY DIE IN SMALL CLUSTERS;
- YOUNG TREES ARE MORE LIKELY TO BE AFFECTED BY THE DECLINE THAN OLDER (OVER 7 YRS.), AND SHOOTS ARISING FROM SHORT CUT STUMPS ARE OFTEN ALSO AFFECTED.

B. ELECTRON MICROSCOPIC ANALYSES AND OTHER CONSIDERATIONS IMPLICATE A SOIL-BORNE FUNGUS WHICH CAUSES A SO-CALLED VERTICILLIUM PERMANENT WILT. BASED ON THE PATTERN OF DISEASE SPREAD OBSERVED, TRANSMISSION MAY OCCUR VIA PLANT-ROOT INFESTING NEMATODES, OR THE DISEASE AGENT MAY BE MECHANICALLY TRANSMITTED IN SOIL. INITIAL SUSPICIONS REGARDING A VIRAL CAUSATIVE AGENT HAVE NOT BEEN CONFIRMED BY THE LABORATORY STUDIES CONDUCTED TO DATE. THIS IN ITSELF LENDS A MORE HOPEFUL PROGNOSIS TO THE LIKELIHOOD OF SUCCESS OF AN APPROPRIATE STRATEGY TO COUNTER THE DISEASE IF IT IS INDEED FUNGAL IN NATURE. DIAGNOSTIC TESTS AND INTERVENTION STRATEGIES FOR FUNGAL INFECTIONS ARE READILY AVAILABLE, AND RIGOROUS PREVENTIVE AND PLANT HYGIENE MEASURES CAN GO A LONG WAY TOWARDS STEMMING THE SPREAD OF FUNGAL DISEASES. WITH A VIRAL DISEASE, OPTIONS WOULD BE FEWER.

3. WHILE FOLLOW-UP AND REMEDIAL ACTION STEPS ARE BEING DEVELOPED, PLEASE NOTE THAT THE PRELIMINARY RECOMMENDATIONS MADE BY DR. BATRA IN HIS FIELD INVESTIGATION REPORT STILL APPLY. FYI: A REVISED UPDATED VERSION OF HIS FIELD REPORT IS BEING POUCHED BY AFR/TR TO ALL SAHELIAN MISSIONS. END FYI.

4. AFR/TR/ANR RECOMMENDS A FOLLOW-UP MISSION, BASED ON DISCUSSIONS WITH FSP, BATRA AND OTHER SCIENTISTS. A SEARCH HAS BEEN INITIATED FOR A FRENCH-SPEAKING EXPERT ON FUNGI AND TREE PATHOLOGY, PREFERABLY A USDA-AFFILIATED SCIENTIST WHO CAN IDENTIFY VERTICILLIUM AND SIMILAR FUNGI AND WHO IS FAMILIAR WITH AGROFORESTRY IN SEMI-ARID CLIMATES.

5. THE FOLLOW-UP MISSION SHOULD ADDRESS THESE MAIN ASPECTS:

A. FIELD STUDY IN NIGER TO CONFIRM THE DISEASE DIAGNOSIS AND EXTENT OF INFECTION, PREFERABLY INCLUDING VISITS TO NEW SITES IN NIGER, AND POSSIBLY INVOLVING TRANSMISSION STUDIES;

B. LAB AND FIELD DIAGNOSTIC STUDIES, WITH COLLABORATORS IN NIGER, AND IN THE U.S;

C. PLANNING APPROPRIATE COMPREHENSIVE SURVEY OF NIGER AND POSSIBLY OTHER SAHELIAN COUNTRIES, INVOLVING FORMAL DISEASE INDEXING TO OBTAIN QUANTITATIVE DATA ON THE EXTENT AND SEVERITY OF THE PROBLEM.

D. PRELIMINARY STRATEGIZING FOR FUTURE TRAINING PROGRAMS DEALING WITH RECOGNITION AND MANAGEMENT OF THE NEEM DISEASE IN NIGER; THIS SHOULD INVOLVE THE KEY NETWORK OF INTERESTED GON, DONOR AND NGO/PVO PARTIES.
FROM SUCH DISCUSSIONS PRESUMABLY WILL EVOLVE USAID/NIGER'S APPROACH TO DEALING WITH THE NEEM DISORDER, POSSIBLY AS PART OF ITS AG SECTOR DEVELOPMENT PORTFOLIO.

6. FOR CONFIRMATION DIAGNOSTICS, FRESH MATERIAL WILL BE NEEDED. THE QUALITY OF THE ORIGINAL SPECIMENS AND SOIL SAMPLES RETURNED TO THE U.S. WAS NOT OPTIMAL, PARTLY BECAUSE THE SHORT LEAD TIME FOR DR. BATRA'S TRIP DID NOT ALLOW PROPER USDA/APHIS QUARANTINE PERMITS TO BE OBTAINED. AS A RESULT, THE RESULTS OF THE LAB TESTS WERE COMPROMISED SOMEWHAT. SEVERAL APPROACHES TO OVERCOME THIS PROBLEM ARE PROPOSED FOR THE NEXT VISIT:

A. OBTAIN NEEDED USDA/APHIS PLANT PROTECTION AND QUARANTINE PERMITS WELL IN ADVANCE;

B. PRIOR APPROVAL BY APHIS OF THE RECEIVING LABORATORY(IES);

C. PREPARE AND FIX SPECIMENS ON SITE (AS APPROPRIATE FOR TRANSMISSION ELECTRON MICROSCOPIC STUDY, FOR EXAMPLE), FOR RETURN TO THE U.S. AS PRESERVED SPECIMENS, WHICH ARE NOT SUBJECT TO QUARANTINE RESTRICTIONS;

D. IDENTIFY LABORATORIES IN NIGER WHICH CAN ASSIST WITH ON-SITE DIAGNOSTICS (MAINLY TO PROVIDE LABORATORY SPACE AND INCUBATION FACILITIES).

7. IN REFERENCE TO PARA 6D ABOVE, CERTAIN KEY STEPS TO THE DIAGNOSIS OF PLANT DISEASES CAUSED BY FUNGI COULD BE RELATIVELY READILY ACCOMPLISHED WITH MODEST LABORATORY SUPPORT, PARTICULARLY IF THE SPECIALIZED CULTURE MEDIA ARE BROUGHT ALONG. ESSENTIALLY ALL THAT WOULD BE NEEDED ARE: LAB COUNTER SPACE, AN INCUBATION FACILITY, AN AUTOCLAVE OR OTHER MEANS OF STERILIZING BY STEAM, AND MODEST AMOUNTS OF COMMON LAB SUPPLIES AND EQUIPMENT.

8. ACTIONS REQUESTED OF USAID/NIGER:

A. IDENTIFY A LABORATORY WHICH COULD COLLABORATE WITH THE SPECIALIST PROPOSED TO BE SENT. POSSIBILITIES ALREADY IDENTIFIED ARE, IN ORDER OF LIKELY AVAILABILITY: DFPV/AGRHYMET (MS. COREY BROOYMANS, PLANT PATHOLOGY LAB ASSISTANT); INRAN (MR. HASSANE HAMA, PHYTOPATHOLOGY AND ENTOMOLOGY LAB); UNIVERSITY OF NIAMEY, PLANT PATHOLOGIST; AND THE ICRISAT SAHELIAN CENTER (DR. HESS, PLANT PATHOLOGIST).

B. IDENTIFY APPROPRIATE COUNTERPARTS TO SUPPORT AND ACCOMPANY THE SPECIALIST. FYI: FSP HAS CONTACTED REMCO VONK, OF CARE INTERNATIONAL, NEW YORK, AND OBTAINED CONCURRENCE FOR MAMADOU DIAKITE, REGIONAL TECHNICAL ADVISOR FOR CARE IN NIGER, AND OTHER CARE STAFF, TO BE INVOLVED IN SUPPORTING THE FOLLOW-UP VISIT TO THE EXTENT APPROPRIATE. END FYI.

C. IDENTIFY RECOMMENDED TIME PERIOD AND LENGTH (14 DAYS IN-COUNTRY MINIMUM RECOMMENDED) OF A FOLLOW-UP DURING THE MONTHS OF OCTOBER TO DECEMBER 1991.

D. CONFIRM THAT A VEHICLE, MISSION SUPPORT, AND IN-COUNTRY PER DIEM CAN BE PROVIDED BY USAID.

9. OTHER ADDRESSEE MISSIONS MAY WANT TO BRING THE APPEARANCE OF THIS DISEASE IN NIGER TO THE ATTENTION OF APPROPRIATE AUTHORITIES IN THEIR COUNTRIES, IN THE EVENT THAT SIMILAR PROBLEMS HAVE BEEN NOTED ELSEWHERE. AS STATED ABOVE, THE REPORT IS BEING POUCHED.

10. KINDLY DIRECT YOUR RESPONSE, WITH ANY ADDITIONAL INPUT AND SUGGESTIONS YOU MAY WISH TO MAKE, TO WALTER KNAUSENBERGER, AFR/TR/ANR/NR (SOON TO BE AFR/ARTS/FARA/NR AND ENV), CARL GALLEGOS/BOB MOWBRY, S&T/FENR, AND JULIA MORRIS, USDA/FS/FSP. IF SIMILAR PROBLEMS HAVE BEEN OBSERVED ON NEEM IN THE SAHEL OR ELSEWHERE, THIS WILL BE USEFUL TO KNOW.

YY

VASCULAR DECLINE OF NEEM IN NIGER
(Investigation Report)
Amended Report No. 278-9205 A

Submitted To

Dr. Walter I. Knausenberger
U.S. Agency for International Development
Africa Bureau
TR/ANR/NR
Washington, DC

By

G.K. (Paul) Batra, Ph.D., Plant Pathologist
Biosystems/Atlanta
762 U.S. Highway 78
Loganville, GA 30249
Telephone (404) 466-1511
Fax (404) 466-1512

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VASCULAR DECLINE OF NEEM IN NIGER

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VASCULAR DECLINE OF NEEM IN NIGER

EXECUTIVE SUMMARY

A vascular disorder of neem, tentatively considered to be caused by Verticillium sp., as a result of this field investigation is reported from Niger, West Africa. The disease has the following characteristic symptoms: 1. Young leaves change color from a shiny green to a dull olive green or a pale yellow. 2. Leaves are often deformed, crinkled, curled, rosetted, in-folded, and compressed together near branch tips. Older leaves fall off early. 3. Shortened internodes near apex coupled with scars from fallen off leaves toward the base of the branch give it a bunchy, bushy, and spindly appearance. 4. Denuded shoots often exhibit permanently wilted, yellowed, necrotized, gummy tips. 5. Shiny, clear white to translucently yellow gum exudes from branch tips. 6. Stunting and yellowing of young branches precedes necrosis (a centrifugal die-back of branch and apical tips), leaving behind bare, dead, scorched branches, and eventually, the death of the entire tree results. Young (three-month seedlings to 5 year-old) trees are more likely to be affected than older (7-year +) trees. Young adventitious shoots arising at the base of a short stump (a tree cut <0.5 m from ground) are similarly diseased. The vascular decline of neem appears to be prevalent throughout Niger, and its incidence can vary from less than 10% of trees in a mature (over 12 years old) plantation to over 75% in a younger (less than 5 years old) plantation. Based on signs, symptoms, epidemiology, and laboratory studies, including electron microscopy observations, the suspected pathogen appears to be a fungus, Verticillium sp. The mode of transmission is unclear, but appears to be via soil. This disease appears to be on the rise in Niger, and on account of its ability to devastate large plantations, may become the determining factor in success/failure of neem planting programs in Niger and other surrounding Sahelian countries. Due to the great economic importance of neem, it is imperative that this disease be studied further to confirm its suspected etiology, mode of transmission, and possible control measures as soon as possible. In the meanwhile, tentative recommendations are to cut and burn infected trees, sterilize soil and mechanical tools for cutting or pruning trees, and stop further plantings, particularly of a monoculture type, until a suitable fungicide is identified, and/or disease-free or disease-resistant cultivars are available.

VASCULAR DECLINE OF NEEM IN NIGER

1.0 BACKGROUND

An incidence of a systemic disease of neem was initially reported in Sani-Tanko village near Gadabeji, 80 km from Dakoro, Niger in November 1990 to Gerard PJM van der Burg, Agroforester with Care International in Tahoua (Knausenberger, 1991). Apparently, 4-5 neem trees in a group on a farmer's property had died within a few months. Symptoms included gummosis from fissures in bark, yellowing of sapwood, die-back of twigs, and death of the tree within a few weeks. Internodal distance on young twigs was shortened and leaves were compressed together. No primary insect infestation was indicated. Since 1990 was a low rain year, it was suspected that drought may have stressed the plants, and that the symptoms observed may have been stress-related. The major concern was that if it were an infectious disease it might spread to the neighboring Maggia Valley Windbreak Project plantation consisting of 1.5 million neem trees, and jeopardize the success of that project. Samples were collected on January 22, 1991 and processed for isolation of bacterial and fungal pathogens at plant pathology laboratory of DFPV at AGRHYMET in Niamey, Niger. Unfortunately, samples collected were from dead trees, which were "bone-dry", and were mistakenly frozen on receipt. Therefore, subsequent culturing yielded nothing but a few surface bacterial contaminants.

In his memo of 31 January 1991, Dr. Walter I. Knausenberger, U.S. Agency for International Development, recommended that a field investigation be conducted by a trained tree pathologist with experience in arid regions, and that a new set of samples be obtained and appropriately processed to diagnose the disease. Dr. G.K. (Paul) Batra, plant pathologist, Biosystems/Atlanta was selected on June 20, 1991, to conduct a field investigation. Dr. Batra left Atlanta on June 22, 1991, and arrived in Niamey, Niger on the evening of June 24, 1991. Field investigation began on June 25, 1991 and ended on July 7, 1991, and Dr. Batra returned to Atlanta on July 8, 1991. Components of this report were submitted as Field Investigation Report No. 278-9205 on July 15, 1991 and as Laboratory Analysis Report No. 278-9206 on August 14, 1991 to the sponsors.

2.0 INTRODUCTION

2.1 Neem

Neem or nimba (Azadirakhta indica, family Meliaceae), a relative of mahogany and bakayan (Persian Lilac), is originally a native of India, and is now habituated in most of South and Southeast Asia, viz. India, Andaman Islands, Pakistan, Sri Lanka, Burma, Malaya, Indonesia, Japan, as well as some tropical regions of Africa and Australia. Neem is an evergreen tree growing to a height of 40-60 feet, has a straight trunk and long spreading branches forming

a broad round crown or canopy of leaves at the top. Some excellent monographs are available on its botany and utilization (Mitra, 1963; Ketkar, 1976). The tree sheds its old leaves in February-March and produces young, glossy green leaves and fragrant white flowers in April. The wood is hard, colored, and odorous. The leaves are compound, imparipinnate, alternate, exstipulate, 22-37 cm long, borne on long slender petioles. Leaflets are 7-17 in number, alternate or opposite, very shortly-stalked, 6-7.5 cm long, ovate-lanceolate, attenuate at apex, unequal at base, upper half much longer than the lower, coarsely or bluntly serrate, smooth, and dark green in color. Flowers are hermaphrodite, small, shortly-stalked, panicled, and with deciduous bracts. Calyx: 3-5 sepals, wide and spreading, imbricate, rounded, blunt, ciliate, smooth, thin. Corolla: 5 petals , white, imbricate and oblong, oval in bud, 4-5 mm long when mature, oblong, spathulate, somewhat twisted and with a conduplicate claw, smooth outside, finely pubescent within. Androceum: 10 stamens, at the base of hypogynous disc, staminal filaments combined into long, cylindrical tube, furrowed, smooth externally, pubescent within, terminating above in 10 blunt, thick, recurved, irregularly trifid lobes; anthers small, erect, closely placed, oblong, two-celled. Gynaeceum: Tripentacarpellary, syncarpous, superior, two ovules in each locule, style about the length of staminal tube, stigma 5-lobed. Fruit is a drupe. Gametophytes develop normally and embryo sac is formed after polygonum type. The seed is exalbuminous and germination is epigeal.

Neem is a fast growing, hardy tree, growing best in semi-arid tropical regions with a rainfall of at least 450 mm annually (Hegde, 1990). It is a moderately salt-resistant tree and grows successfully in poor soils (rock, sand or clay), including dry, shallow, saline, or alkaline soils, even in extreme drought, but will not thrive in areas where frost may occur, or in soils with high moisture, in water-logged conditions, or in areas with poorly drained soils and an average annual rainfall exceeding 1200 mm (Ritchie, 1990). It is this quality of hardiness, perhaps the most important attribute of neem, which, along with its value as a shade tree and as a biological control for soil-erosion and wind-erosion, has allowed its successful introduction in early 20th century to Pakistan, Sri Lanka, Thailand, Indonesia, the Middle East, and parts of Africa.

In Niger (Maps 1-3), "The Neem Story" began on several, initially separate fronts, through the efforts of Deutsche Gesellschaft fur Technische Zusammenarbeit (GTZ), Nigerien Ministry of Agriculture, Department of "Protection des Vegetaux", Care International Au Niger, U.S. Agency for International Development (USAID), and Government of Netherlands Directorate General of International Cooperation - Netherlands (DGIS), and came to a collaborative, generally well-coordinated climax, typified by a world-wide three-

day Workshop on neem in 1989 in Niamey (G.P.J.M. van der Burg and K. Hassane, 1990). Perhaps, of all plots of the neem story in Niger, none has succeeded more, or is as well known as the Maggia Valley Wind-break Project near Tahoua where over 1.5 million neem trees in series of rows were planted across the Maggia valley to serve as windbreaks and to stem the severe soil erosion caused by wind, and now farmers are able to grow valuable grain crops of millet and sorghum in the area, (Kerkhof, 1990). In general, neem is well-established and is fairly densely populated (up to a density of 8,000 - 500,000 trees per arrondissement (district) in most of southern Niger, where annual rainfall ranges from 400 to 600 mm (Maps 4-5).

2.2 Economic Importance of Neem

Neem is an excellent example of a plant with a truly high material value and general usefulness for human society, and thus has a great economic significance, and must command at least an honorable mention in any economic botany text. The tree as a whole, individually and severally in communities, and all parts of the tree are useful. Many of these uses are derived from neem's pesticidal properties. The tree is valued as an important shade tree, particularly in hot, dry, sun-parched, arid and semi-arid areas of the world where nothing else seems to survive due to high heat, low moisture, and poor soils. As a vibrant, living green plant in the middle of a dry, drought-stricken, dismal, desolate, and seemingly dead Sahel, it has an aesthetic appeal unmatched by any other object in the landscape. It literally serves as an oasis for life where butterflies, birds, and small animals take shelter in the middle of the desert-like Sahelian environment, perhaps one of the most inhospitable, hostile, and foreboding environments on the planet Earth.

Neem wood is used both as timber and as fuel. Relatively straight branches are excellent as poles for fencing and roofing, and their resistance to termites and other wood-destroying organisms is a welcome attribute in warm climates where such pests can be very active and destructive. Neem wood is also suitable for fabrication of agricultural implements, but less suitable for furniture as it has an offensive odor. Neem makes excellent firewood due to its high calorific value, and is a renewable source of fuel since it has a high annual growth rate. Offensive fumes limit the use of neem wood indoors, but charcoal made from neem is considered of high quality. Neem leaves are used as fodder for animals, including cattle, goats, sheep, etc., and have a high nutritional value. However, due to the bitter taste of neem leaves, animals eat them as a last resort. Therefore, neem leaves have a rather limited utility as primary feed, but as a feed supplement they add valuable nutritional elements to dry fodder (Shukla and Desai, 1988). Neem leaves are also known to combat worm-infestation in

livestock. Neem leaves are also used as a soil adjuvant for soil improvement, as a mulch to enrich poor soils, and to regulate soil humidity. Leaves are used as a pesticide-manure in Sri Lanka. Another use for neem leaves is for protecting stored grains from various pests. Neem leaves, either as whole or as chopped, are mixed with grains in storage. Apparently, the offensive odor of neem drives insects away. Similarly, green neem leaves are used by roadside meat vendors to drive away flies from meat, and dry leaves are burnt around homes to repel flies, mosquitoes, and other flying insects (Ritchie, 1990).

Bark and twigs of neem are important ingredients of several indigenous medicines and drugs effective for septic wounds, ulcers, skin diseases, stomach worms, and muscular aches and pains in the practice of traditional medicine, Ayurveda, in India and Sri Lanka. Neem twigs, with one end of the twig chewed into bristles, are the preferred tooth brush for cleaning teeth and gums in India and adjoining countries. Neem seeds have the highest concentration of the active pesticidal ingredient, azadirachtin, are processed in different ways (crushed with a pestle and mortar or extracted with various solvents), and yield a variety of products in three basic categories: neem powder, neem oil, and neem cake. Neem powder is produced by simply crushing the neem seeds with a pestle and mortar. Powder can be applied directly in the form of a dust for its pesticidal value, or it can be extracted with water to prepare an aqueous solution for spraying. Neem oil is extracted by pressing neem seeds through a mechanical press and is useful as a medicine, pesticide, lubricant, lamp oil, and for making soaps, toothpaste, and cosmetics. Neem cake is a by-product of the oil extraction process, and is used as a fertilizer, mulch, and as animal feed supplement.

Neem is an important tree species in afforestation programs to ameliorate the damage caused by soil erosion in most of the Lake Chad Basin, and represents 80% of seedlings supplied by nurseries (Lake Chad Basin Comission, 1989). This is primarily due to hardiness of the plant in that the tree can grow in poor soils and under daunting climatic conditions, grows fast, and develops an excellent network of roots which hold the soil well together in the form of a ball, thus resisting soil erosion. Neem tree has been successfully deployed as an effective biological wind-break mechanism to slow down the dry, hot, mechanically desiccating and shearing winds from the Sahara, and thus slow down the wind erosion of top soil in Maggia valley in Niger and elsewhere (Kerkhof, 1990). The use of various neem products as biological pesticides has been addressed above, and is based on the presence of various antibacterial, antifungal, and insecticidal as well as insect-repellent properties of its biologically active constituents: nimbin, nimbinin, nimbidin, desacetylnimbin, nimbinic acid, neonimbidin, 'Neutral D', nimbidinin, and azadirachtin (Mitra,

1963). Perhaps, the value of neem as a pioneer plant in the establishment and succession of plant communities in arid and semi-arid areas has not been fully realized and utilized. As mentioned earlier, in the hostile environment of Sahel, perhaps no other plant species survives and thrives as well as neem, and holds the eroding soil together, provides the rich humus and other organic constituents to the poor soil, increases its water-holding capacity, and thus allows the operation of a natural soil conditioning program for successive plant (and animal) communities to follow on an otherwise barren land.

2.3 Diseases of Neem

The very thought of a neem disease appears antithetical since the plant is considered synonymous with cleanliness and health due to its pesticidal properties. It is not surprising, therefore, that the monographs referenced above and other published literature do not even hint the report of a single neem disease. To our knowledge, only two known neem diseases (if we can call them that at this point) are known to date. The first is a cochineal scale (Aonidiella orientalis) infestation as noted in the cochineal infestation control project proposal (Lake Chad Basin Commission, 1989). The second is this suspected viral disease, the topic of this report's discussion.

2.3.1 Literature review

Prior to proceeding with the field evaluation, Dr. Batra, utilizing the data bases of USDA'Agricola (AGRICOLA) and Commonwealth Agricultural Bureau (CAB) at the University of Georgia, conducted a preliminary search of literature for published reports on diseases of neem. While there were a number of published reports on the pesticidal properties of neem during the period 1987-1991, there was not even a single report of a disease of neem during this period, except for a report about the fungal flora of neem seeds (Sinniah et al. 1983). While this preliminary search does not purport to be an exhaustive one, it tends to support the notion that neem does not have too many pathogens or pests, and seems to further reinforce the theme that the plant has pesticidal or pest-resistant properties.

There is a mention of a cochineal scale or oriental scale (Aonidiella orientalis) infestation of neem, apparently first noticed in 1972 in a fruit tree nursery in Garoua, Cameroon where a second infestation was noticed again in 1984, and a third incidence in Banki, Nigeria, in 1987, in the cochineal infestation control project proposal (Lake Chad Basin Commission, 1989), but no references were included in the copy of a pre-print made available to us. Apparently, a great deal of damage was caused by the scale of which three species in Cameroon and two in Nigeria

were noted. A number of control measures, short-, medium-, and long-term such as cutting and burning, chemical treatment (dimethoate, chlorpyrophos ethyl, feunitrotion (sic), and aldicarb (Temik)), oil-fogging, biological control, and genetic selection were suggested. Nothing further is known about the infestation since original reports are unavailable at this time. Based on an Informationcentre for Low External Input and Sustainable Agriculture (ILEIA) newsletter (March 1990), there is another reference to this scale infestation on neem in the Lake Chad area in a news report (Anonymous, 1990), which suggests that the scale is often associated with neem, becoming a pest problem only when plants are stressed leading to an outbreak after drought conditions. It also suggests that scale insect obviates the pesticidal effect of azadirachtin by feeding on phloem tissue which is essentially devoid of the natural pesticide. We presume that the neem yellow scale problem in southeast Niger, northern Cameroon, and northern Nigeria (Lake Chad Basin) referred to in the Scope of Work for this project assignment is the same scale problem discussed above.

About the time (late June - early July, 1991) I was in Maradi, Niger, there were anecdotal accounts of a neem disease, with symptoms similar to the one investigated in this report, being rampant in northern Nigeria. Similarly, there was some speculation regarding the mechanical transmission of the disease into southern Niger via tall freight trucks, tops of which often sweep beneath the lower canopy of neem trees growing on the roadside. While it stands to reason that a viral disease could be transmitted in this manner, it would have to be a highly stable virus like tobacco mosaic virus to withstand inactivation at high temperatures, desiccation by hot, dry winds, and wind shear of high truck speeds. We had no occasion to go to northern Nigeria to observe this situation for ourselves, and we did not see many large trucks sweeping beneath neem trees on roadside in Maradi or southern Niger. Roads with neem trees that we observed were fairly wide and, therefore, there would be rather a small probability of the trucks touching the trees, and thus transmitting the disease from tree to tree.

3.0 MATERIALS AND METHODS

3.1 Field Investigation Chronicle

3.1.1 Niamey Area

Monday, 24 June 1991

Observed several adult neem trees (10-15 years old) on the roadside, in the vicinity of Hotel Sofitel and Palace De Congress. Apparently healthy trees, flowering and bearing fruit. No overt

signs or symptoms of a disease, localized on any part of the tree or systemic. No insect infestation.

Tuesday, 25 June 1991

During mid-morning, I held technical discussions with Hamadou Bourahima, Barry Rands, Meg Brown, and Stephen Haines at USAID in Niamey, and I was able to utilize library facilities in Barry's office. Accompanied by Hamadou, I visited the Office of Director General, Niger Dept of Environment, and held fairly broad discussions on the subject with Mr. Alio Hamidil, Ingenieur des Eaux et Forets, Directeur National de l'Environnement, Niamey, Republic du Niger. He expressed a keen interest in the investigation and a great concern regarding the potential impact of disease on neem plantation programs in Niger.

In the afternoon, I visited Madame Corey Brooymaus, Senior Technician at Plant Pathology Laboratory, Agronomy, in Departement de Formation en Protection des Vegetaux (DFPV) in AGRHYMET complex in Niamey, who had attempted to isolate microbial pathogens from sections of diseased neem stem and leaf tissues collected earlier. The laboratory was well-equipped, organized, and maintained for microbiology and plant pathology work. "Madame Corey", as she is often known in those parts, and her assistant, both of whom were very cordial, appeared to be extremely competent and enthused about their work. They showed me several petri dishes containing malt extract agar and potato dextrose agar with embedded or superficial brownish samples of neem tissues. Apparently, the cultures were 7-10 days old and showed grayish white, translucent to hyaline, bacterial colonies surrounding the samples, perhaps of a contaminant such as Bacillus subtilis, often encountered in the laboratory. Madame Brooymaus agreed to my suggestion that the bacterial colonies on media probably represented contaminants. On my questioning, she affirmed that samples were surface-sterilized by a dip into 10% sodium hypochlorite for two minutes, however, no antibiotics were incorporated into the medium to suppress bacterial contamination. She also agreed with me that she did not expect any microbial isolates since neem specimens on receipt, prior to culturing, were mistakenly frozen at -12 F. This mistake probably occurred due to mis-communications regarding the nature of samples and the purpose of testing, according to Madame Brooymaus. While in the laboratory, I also examined a specimen of a trunk from a diseased neem tree with dried branches and leaves. A cross section of stem showed extreme yellowing of sapwood and some gummosis at the periphery of trunk.

I also visited Madame Denise of CIDES (Documentation Center), Ministry de Plan, in an effort to obtain any references or published reports on neem diseases in the area. She informed me that they have a database on socio-economic statistics in Niger,

but nothing on scientific and technical matters. She suggested that I might try library facilities at University of Niamey or at International Crops Research Institute for the Semi-Arid Tropics (ICRISAT). I was unable to contact libraries at University of Niamey and ICRISAT, and Dr. Hess, plant pathologist at ICRISAT was out of town for an extended period of time.

[Note: Niamey area chronicle continues after Tahoua area observations].

3.1.2 Maradi Area

Wednesday, 26 June 1991

Accompanied by Hamadou and his driver Hassan, I travelled a distance of approximately 650 km in a USAID vehicle from Niamey to Maradi. I observed a number of apparently healthy adult neem trees on the roadside and in courtyards of homes by the roadside; also observed some wind-breaks by the roadside. There were no visual signs of distress, although, occasionally, some of the trees were observed to be missing in a row or stunted in growth.

Thursday, June 27, 1991

I visited the Office of the Area Director of Environment, Maradi where I met two of his associates. Mr. Edigouini was to accompany us for our field trip to Mayahi planned for the next day. The area director was unavailable for discussions, so we planned to return later in the afternoon. At the Office of Area Director of Environment, I also met a bright peace corps volunteer from Boston, Ms. Suzanne Reasonberg, who showed us her prized collection of seeds of several local species of Acacia and other indigenous plants that she distributes to farmers in the area for their agro-forestry operations, primarily for fencing and border plantings.

I visited the Offices of Care International, Maradi where I had the pleasure of meeting Mr. Leigh Heart, Directeur, Care International Au Niger, Maradi, and Mr. J.P. (Hans) Van Kooyck, Coordinateur of Agro-forestry. Mr. Heart updated me regarding the neem disease situation in the area, and I had the opportunity of reviewing some valuable literature on agro-forestry in general, and neem plantations in particular, made available to me by Mr. Van Kooyck and Mr. Heart. I was greatly impressed by the organization and operations of Care International in Maradi, particularly their interest and achievements in agro-forestry in the area. I again visited the Office of the Area Director of Environment where I had the pleasure of meeting with Mr. Inoussa Seybou, Ingenieur des eaux et forets, Directeur Departmental de L'Environnement, Maradi, a very knowledgeable and genial person. He expressed a keen interest in our work, and a grave concern for the welfare of neem in the

area, and impressed upon us to debrief him with our findings after our field trip to Mayahi the next day.

We visited again the offices of Care International, Maradi where I had some informal discussions with Mr. Heart on neem and its value to Niger and other Sahelian countries, neem diseases (the termite problem on neem and a systemic disease of neem in northern Nigeria) and their implications to neem projects in the area, possible control measures, including biological control. Mr. Van Kooyck was very helpful in making available to me copies of several reports on their agro-forestry operations, including a Care International Au Niger multi-year plan for the period 1990-94. Observed adult (8-10 years old) neem trees on the main road (Maradi-Zinder Road) and airport road in Maradi. All were in excellent health, and I found no hint of a disease, localized or systemic.

(Note: Maradi area chronicle continues after Mayahi area chronicle.)

3.1.3 Mayahi Area

Friday, June 28, 1991

We travelled about 45 km on a surfaced road and another 90 km on a dirt road, and arrived at Mayahi, NE of Maradi, at about 9:00 am where we were received by Mr. Tairigu Toimane (Sp.?), Head of Environment Service, who took us to meet Mr. Abdou Liman, Administrator of Mayahi. The neem plantation in Mayahi consisted of approximately 24,000 trees spread over 60 hectares, with a density of 400 trees per hectare. The plantation is on a generally sandy soil, and is approximately 5-7 years old. We found almost half the trees on the plantation either dead or dying (Figs. 1 - 6). Two spot checks within visual fields showed a 30-50% incidence of affected trees.

Symptoms of the disease are characteristic and include: Young leaves change color from shiny green and violaceous red to a dull olive and pale yellow. Leaves are often deformed, crinkled, curled, rosetted, in-folded, and compressed together near branch tips (spindly). A permanent wilting of an entire branch, usually the apical one, is seen quite often. Older leaves fall off early. Shortened internodes near apex coupled with scars from fallen off leaves toward the base of the branch give it a bunchy, bushy, and spindly appearance. Denuded shoots often exhibit permanently wilted, yellowed, necrotized, gummy tips. Shiny, clear white to translucently yellow gum exudes from branch tips. Stunting and yellowing followed by necrosis leads to a centrifugal die-back of branch and apical tips leaving behind bare, dead, scorched, "bone dry" branches, and eventually, the death of the entire tree

results. Young (three-month seedlings to 5 year-old) trees are more likely to be affected than older (7-year +) trees. Young adventitious shoots arising at the base of a short stump (a tree cut <0.5 m from ground) are almost always diseased, but rarely so when the cut is made high (2-3 m) above the ground (Fig. 2). No mottle or island effect was evident. Two plants, one dead and the other dying, were dug up (Fig. 4) to the tertiary root level, but no root damage or malformation was seen.

No symptoms in the form of any malformation, nodules, cankers, lesions, discoloration, spotting, scars, softness, mushiness, etc. or signs as fungal mycelia, sporangia, spores, sclerotia, other fructifications, or insect egg masses were noted associated with leaves, branches, stem, or roots of the plant, when viewed with the naked eye or through a hand magnifying glass (approximate mag. 5 x). Seven or eight black and yellow striped beetles (approximately 5 mm x 15 mm) were seen foraging on one dying sapling. We collected three beetles, while the others took flight on being disturbed. We did not see again this type of beetle in association with another neem tree in the plantation or anywhere else.

We also collected samples of leaves, flowers, fruits, stem, and roots from both apparently healthy as well as diseased trees. These samples were separately double-bagged in Ziplock bags, labelled, and stored in a backpack kept in the shade in the back of an air-conditioned vehicle during travel, and in a refrigerator afterwards. We also took photographs of the plantation, the damaged trees, and some close-ups of disease symptoms. We did not take any soil samples at this point because we did not see any root damage or malformations due to biota in the soil.

3.1.4 Maradi Area (Contd.)

We examined the freshly collected samples of leaf and shoot tissue from Mayahi after returning to INRAN's Phytopathology and Entomology Laboratories in Maradi, through the courtesy of the resident entomologist, Mr. Hassane Hama. No symptoms in the form of any malformation, nodules, cankers, lesions, discoloration, spotting, scars, softness, mushiness, etc. or signs of fungal mycelia, sporangia, spores, sclerotia, other fructifications, or insect egg masses were noted associated with leaves, branches, stem, or roots of the plant when examined with a binocular microscope (approx. mag 75 x) and with a phase contrast microscope (approx. mag 200 x). We did see a few hyphae appearing to be resident or resulting from a secondary fungal invasion of a leaf from one of the samples examined under the phase contrast microscope. Since we had some time on our hands, we paid another visit to Care International to pick up our copies of reports. Mr. Van Kooyck, in cooperation with INRAN, had organized a seminar on agro-forestry and environment in Maradi on July 3-5, and invited

me to attend, but unfortunately I had to decline since we were scheduled to go Tahoua next day, and besides I had a previous invitation, through the courtesy of Barry Rands, to attend July 4 celebrations with the U.S. Ambassador in Niamey.

3.1.5 Groumdji, Dakoro, and Gadabeji Areas

Saturday, 29 June 1991

We left for a field trip to the Groumdji, Dakoro, and Gadabeji areas. We visited the home of the farmer in village Sani-Tanko near Gadabeji where it all (about the systemic neem disease) began last year, as I understand. I had the pleasure of meeting and speaking with the farmer. We examined the four trees of neem he has in his fenced yard. All trees were affected with the disease with characteristic symptoms, and were in various stages of decline. According to the farmer, plants were 5-7 years old and doing fairly well until about two years back when he noticed a general decline with one of the younger plants. Initially, he too thought the problem was drought-related, but when it did not go away after adequate watering, he suspected it to be a malady of some kind. He became concerned when he observed this problem spread to the other three trees the following year. He had cut one of the trees to the ground, and we observed characteristic disease symptoms on all the offshoots which sprouted from the base of the stump. No, he had not travelled much in the past two years, or done anything unusual to the trees. We removed samples of affected young leaf and shoot tissues, packaged samples in Ziplock bags, and took photographs of symptoms, as noted above (Figs. 7-8).

On our trip back to Maradi, I spotted and examined a fairly large (approximately 250 m x 500 m plantation of neem on the left side of the road in village of Garangsou (Sp.?), near Dakoro. It turned out to be a former USAID Seedling Replication Facility, now under the operations of Niger Department of Environment. It appeared to be relatively a younger plantation (perhaps 3-5 years old). We observed the characteristic symptoms of the disease on most of the plants, but what surprised and shocked me was the large extent of the damage by the disease. More than 75% of the plants were affected, and the plantation was practically devastated, exhibiting localized, contiguous spread of the disease in the field. We removed samples of the leaves and young shoots. We returned to Maradi in the evening.

3.1.6 Tahoua Area

Sunday, June 30, 1991

We travelled by USAID vehicle to Tahoua via Dakoro, and arrived in Tahoua at approximately 3:00 pm. I went to attend the exhibits at

a seminar organized by Organization Nigerienne de Volontaires Pour La Preservation De L' Environnement (ONVPE) at Hotel De Ville. There we met Barry Rands, Meg Brown, and Gerard van der Burg of Care International, Tahoua. Barry invited us to attend ONVPE's banquet in the evening at Hotel L'Amitie. At the banquet I also met Dr. Shiv Kumar from ICRISAT-Niger and Dr. Andrew Manu of INRAN who also were attending the seminar

Monday, 1 July 1991

We visited the office of Mr. P.J.M. van der Burg, R.P.F., Coordinnateur des Projects Agro-Forestiers et Fruitiers at Care International, and briefly discussed our previous findings and our desire to visit Maggia valley plantation with him. He provided us several valuable documents, including copies of the two monographs on neem and several local project reports. Suspecting the disease to be a viral one, I wanted to conduct transmission studies in the laboratory in the U.S. for which I needed neem seeds. Mr. Van der Burg felt that we could get plenty of seeds from Mr. Al Mansour of Department of Environment at Bouza near Maggia valley. Unfortunately, neem seeds are extremely labile, and have a germination period of no more than six weeks, so I worried if they would have any fresh seeds.

We visited the Office of Area Director of Environment, Tahoua, where we met Commandant Issaka Adamou Ounteini, Ingenieur des Eaux et Forets, Direction Departementale de L'Environnement, Tahoua, who accompanied us to Maggia valley via Bouza. At Bouza, we met Mr. Mansour who informed us that he had no seeds. He and his driver in a separate vehicle led us to Maggia valley plantation, just a few minutes away. I inquired if they had seen any disease problem. Mr. Mansour stated that they had no neem disease in Maggia valley. It was an impressive sight: row after row of luscious neem swaying in the gentle breeze, interspersed with millet plantings in the valley (Figs. 9-12). Sure enough, the first couple of rows of neem on the southern end were extremely healthy, without any symptoms of the disease, or a missing tree (Fig.9). However, as we pressed on deeper into the plantation up to a distance of approximately 1.5 km, and to our surprise and dismay, we found several young trees in groups with characteristic disease symptoms as described above. Also, we saw several stumps with young shoots at the bottom similarly affected (Fig 11). I pointed out these diseased trees to our hosts, and they were greatly surprised. I estimated the disease incidence to be between 10 to 20% in Maggia valley plantation.

The confirmed (after Mayahi and USAID plantations) contiguous pattern of the affected trees within a group (Fig. 12) and the diseased shoots from the base of the stumps brought home to me the infectious nature of the disease, and I began to suspect more the

soil involvement in the transmission of the disease, perhaps through nematodes as vectors for the virus. In addition to plant samples, I took two soil samples and collected neem seeds as well. I also decided to go back to Maradi area and take soil samples from either Mayahi or USAID plantation. We returned to Tahoua, and I went back to the seminar where I was able to attend one paper on the effect of delayed rains on crop production by Dr. Shiv Kumar of ICRISAT.

Tuesday, 2 July 1991

Accompanied by Gerard van der Burg and Hamadou, I visited Tahoua Greenbelt Nursery where we observed two groups of 600 neem seedlings each (Figs. 13-14). One group (about 2-4 weeks old) had only primary (cotyledonous) leaves expanded, and no symptoms of the disease, although some leaves were observed to be yellowed, and senescing earlier than others. The second group (12 weeks old) had two to three sets of true leaves, and some plants in a random manner (not contiguous pattern) exhibited characteristic disease symptoms, viz. color change of young leaves, crinkling, and permanent wilting. Incidence was calculated to be 1.5% (nine plants affected out of 600 in the group). We picked up additional copies of reports from Care International, Tahoua. We started back on our trip to Maradi area to pick soil samples. We returned to USAID Seedling Replication Center in the village of Garangsou near Dakoro (Second Trip) and removed two soil samples at the base of diseased neem plants from the plantation.

Wednesday, 3 July 1991

We were ready to hit the road to Niamey, so, I went to say au revoir to Leigh Heart and Hans van Kooyck of Care International, but both were gone to another appointment. Instead, I had the opportunity to meet Mr. Tim Rudkins, a bright young man from Canada, Coordinateur du Project de Creation D'Emplois et des Petites Enterprises with Care International, Maradi. In a short time, he gave me a thumbnail sketch of his efforts at popularizing private enterprise, particularly at small business level, in Niger. He told me about a program to provide credit to small businesses, an item which is sorely needed at grass roots level according to Hamadou. By this time, Leigh also arrived and I was able to debrief him with the field observations regarding the disease, made tentative recommendations, and apprised him of the future work plans.

Left Maradi to travel to Niamey. I observed the disease situation with neem in a windbreak project on the roadside near Groumdji. Plants have characteristic disease symptoms, with incidence rate between 25 to 50%. Similar observations were made at Dakoro on roadside plants, which had similar disease incidence, viz. 25-50%.

3.1.7 Birni 'N Konni Area

At Birni 'N Konni, where we had stopped to get gas at a Shell station, I observed a short neem stump with shoots coming from ground, all with characteristic disease symptoms. At Dosso, observations were made on roadside trees, and a disease incidence of 25-50% was noted. Returned to Niamey.

3.1.8 Niamey Area (Contd.)

Thursday, 4 July 1991

Examined several neem trees on the roadside near Hotel Le Ronier, on Embassy Street and in Rue De Gaul area in Niamey. About 40-50% showed characteristic disease symptoms.

Friday, 5 July 1991

Visited the USAID office in Niamey. Met with Mr. George Callen, Consultant en Commerce International, USAID-Niger, and debriefed him with the neem disease situation in Niger. Met with Mr. George Eaton, Director, USAID-Niger and debriefed him with the neem disease situation in Niger after our field evaluation, made tentative recommendations, and apprised him of the future work plans. Also present were Mr. Stephen Haines, Associate Director, Commandant Moussa Saley, Agroforester with USAID (formerly with Government of Niger), Meg Brown, and Barry Rands.

I made an overseas call to Dr. Singh at USDA-APHIS-PPQ trying to find the status of our plant material import permit application. Permit had not yet been issued, and Dr. Singh would not make any commitment since he felt that the application was incomplete in the sense that both Biosystems/Atlanta and University of Georgia facilities had not been inspected and approved for this type of work in the past. Went to see and debrief Mr. Alio Hamidil, Ingenieur des Eaux et Forets, Directeur National de l'Environment, Niamey, Republic du Niger in his office regarding the neem disease situation. Unfortunately, he was out of town on official business, and I saw and debriefed his assistant, Mr. El Hadji Mahamane Lawali, Ingenieur des Eaux et Forets, Chef due Service de Reboisement, Direction de L'Environnement. I returned to USAID offices and met with and debriefed Mr. Charles Tapp, Directeur, Care International Au Niger on disease situation and my tentative recommendations. He suggested that we should also sample the seedlings at Tahoua Beltway Nursery, and made arrangements with Gerard van der Burg to send representative samples of diseased and healthy seedlings from nursery to me at Hotel Le Ronier in Niamey.

Saturday, 6 July 1991

Examined neem trees in the compound of the Rec Center; no disease problem was seen. Of particular interest was one tree (the only such tree seen by me in Niger) which had been cut but at a high level (2-3 m) above the ground. It exhibited several young shoots sprouting from the base, all of which did not show disease symptoms! Here, it is important to note that all the other trees in the group were not diseased either, so, the absence of the disease in the shoots from the base of tree stumped high may not be that significant. I received via a courier six seedlings with soil (three presumed diseased and three healthy), in properly packaged and labelled Ziplock bags from Gerard van der Burg in Tahoua.

3.2 Laboratory Tests

Samples of plant and soil materials collected on-site were brought to the U.S., cleared through the U.S. customs and quarantine, and laboratory tests were conducted at Biosystems/Atlanta and University of Georgia as follows:

3.2.1 Samples and Quarantine

As mentioned above (3.0 Field Investigation Chronicle), twelve samples of neem shoots, leaves, flowers, and seeds along with soil were collected, and stored at 5-10 C, except during travel when they were stored at ambient (20 C) in the shade from June 28, 1991 to July 5, 1991. Samples were in the custody of U.S. Department of Agriculture, APHIS in New York (JF Kennedy airport) from July 8, 1991 to July 16, 1991 for quarantine purposes. Handling and storage conditions of samples while in the custody of APHIS are not known. Three unidentified beetles collected from a dead neem plant were destroyed by USDA before the release of samples.

3.2.2 Soil (Nematological) tests

Soil from both presumed healthy and presumed diseased plants was examined for the presence of plant pathogenic nematodes in the laboratory of Dr. William Powell, Department of Plant Pathology, University of Georgia, Athens, GA. Examinations were made to rule out the presence of the following nematodes: root-knot, sting, lance, reniform, lesion, stubby-root, ring, stunt, spiral, dagger, sheath, cyst (female and larvae), and cystoid.

3.2.3 Microbiological tests

Standard plate counts (SPC) of plant tissue were determined at Biosystems/Atlanta by homogenizing plant tissue in sterile, 0.001 M phosphate buffer, pH 7.2, in a blender, and plating out on

tryptose soy agar (TSA). Cultures were incubated at 35 C for two days. Similarly, yeast and mold counts (Y&M) were determined by plating out homogenate obtained as above on potato dextrose agar (PDA), pH 3.5, acidified by tartaric acid, and incubating at 25 C for five days (Speck, 1976).

3.2.4 Electron Microscopy

Sections of leaves, stems, and roots from presumed healthy and presumed diseased plants were cut, fixed, and stained, and examined by electron microscopy in the laboratory of Dr. Charles Mims, Department of Plant Pathology, University of Georgia, Athens, GA.

4.0 RESULTS AND DISCUSSION

4.1 The Disease

The disorder appeared to be systemic and vascular in that symptoms were uniform and generalized from plant to plant as well as within the parts of a plant, although symptoms are primarily evident in the young shoots and leaves at the crown of the branches of relatively younger trees. The local officials earlier thought the affliction was drought-related because of poor rains last year, but when I pointed out to them that this year was a normal rainy season and that plants should have recovered from the stress, the localized, contiguous pattern of distribution of the affliction, and the characteristic disease symptoms, they also appeared agreeable to the tentative conclusion that it was a disease rather than a climatic stress response. No herbicides of any kind had been used in the plantation as far as they knew. After symptomatological, epidemiological observations made in Maradi and Tahoua areas, and laboratory studies in Atlanta area, I am tentatively concluding this systemic disorder of neem to be an infectious, vascular disease caused by a fungus, subject of course to confirmation by additional studies.

4.2 Symptomatology:

Characteristic symptoms, seen primarily on the young shoots and leaves at the crown of the branches of relatively younger trees, include general deformation, crinkling and curling of younger leaves, and stunting of young branches, and of dwarfing the tree in general. Initially, the young leaves turn dark violaceous red to dull olive green, later changing to yellow, crinkling and curling, ending in general necrosis, and death. This leads to permanent wilting, and die-back of the entire branch, ending in the death of the tree. No mottle, or island effect was evident. No lesions, or spotting, scars, fungal spores or mycelia were noted, except for a few hyphae appearing to be resident or resulting from a secondary invasion of a leaf from one of the samples examined

under phase contrast microscope in Mr. Hassane Hama's Phytopathology Laboratory at INRAN in Maradi.

4.3 Epidemiology

Disease Prevalence

The disease is fairly widely prevalent geographically. We travelled in and around cities of Niamey, Dosso, Birni 'N Konni, Dakoro, Tahoua, and Maradi, a distance of over 2,500 km, where most of neem is grown in southern Niger.

Disease Incidence
The disease incidence varies from none (0%) to high (75%+). This assessment is based on observations made in several different locations as reported earlier.

Pattern of Disease Spread

Wherever a diseased neem tree was observed, usually there were other trees, similarly diseased, to be found in the vicinity. Usually, the diseased trees were adjacent to one another, in a contiguous manner, suggesting an infectious nature of the disease. Further, the disease symptoms tended to be localized within a given population rather than generalized or random, from the standpoint of a spatial distribution. Younger trees tended to be diseased more often than older, more established trees. This suggested that either older trees developed an immunity of some kind, or that the infection, when it occurred in young trees, stunted further growth and made them appear undeveloped. Alternatively, it may also be interpreted to mean that the disease has arrived on the scene relatively recently. Finally, there was a consistent association of the disease with young shoots arising from the base of a tree trunk cut (stump) close to the ground, whenever disease was observed in the area. The significance of this observation is not yet clearly understood, except that a soil transmission of the pathogen may be involved, particularly, in view of the localized, contiguous disease spread noted above.

Extent of Disease Damage

No formal disease indexing was done so a quantitative extent of the damage by the disease cannot be provided nor can an economic loss value be placed at this time. The disease severity was noted as none, low, moderate, or severe which when considered along with disease incidence can provide a fair measure of the extent of the damage. Table 1 provides disease incidence, disease severity, and other epidemiological data for locations visited.

4.4 Laboratory Studies

Results of laboratory tests with samples obtained in the field are presented in Biosystems Laboratory Analysis Report 278-9206 submitted to the USDA and the USAID on August 14, 1991 (included as Supplement A in this report).

Nematode analysis

Four representative soil samples, one each from Maggia Valley plantation and USAID Replication Center, and two from Tahoua Beltway nursery (one each containing a presumed healthy plant and a presumed diseased plant) were examined for the presence of plant pathogenic nematodes. The results of this examination are included as items 9206-1 through 9206-4 in the above-referenced laboratory analysis report. Except for the soil sample from USAID Seedling Replication Center, which had 28 ring nematodes/ 100 g of soil, all samples lacked plant pathogenic nematodes specified above.

Microbiological analysis

Eight samples of neem plant material, including seeds and leaves from presumed healthy and presumed diseased plants, were tested for standard plate count (bacteria) and yeast-mold count (fungi), and results are included as items 9206-5 through 926-12 in the above laboratory analysis report. A higher number of micro-flora was isolated from neem seeds than that from neem leaves. Thus, 87,000 colony forming units (cfu) of aerobic bacteria were obtained from one gram of neem seeds, while only 58-203 cfu/g were obtained from neem leaves.

Both bacteria and fungi counts from presumed diseased leaves were significantly higher than those from presumed healthy leaves. For example, SPC obtained from presumed healthy leaves from USAID Replication Center were 132 cfu/g, and SPC from presumed diseased leaves from the same location were 235 cfu/g. Similarly, Y&M counts from presumed diseased leaves from the above location were 193 cfu/g while those from healthy leaves from the same location were only 100 cfu/g. A similar pattern of increased number of micro-flora in diseased leaves was also observed in specimens from Maggia Valley plantation.

Electron Microscopy analysis

Twelve samples of neem plant material were submitted for processing and examination by electron microscopy. Examination of sections from most specimens, typified by the diseased specimen from Tahoua Beltway Nursery (Item 9206-4 in above-referenced laboratory analysis report) revealed no virus particles or inclusions of any kind. The electron microscopy examination, however, showed intra-

cellular fungal hyphae, which appeared to be similar to those belonging to the fungus Verticillium sp. in the vascular bundles of leaves and young shoots.

4.5 Etiology (Suspected)

As indicated above, symptomatological and epidemiological data obtained from field investigation coupled with laboratory studies, suggest that a bacterium, a virus, or an insect is not consistently associated with the disease. A soil-inhabiting fungus, such as Verticillium sp., on the other hand, is a strong possibility. From the general pattern of the incidence and spread (localized) of the disease, nutritional stress as the causal agent also may be ruled out. Further, since no herbicides have reportedly been used, herbicide injury may also be ruled out. Therefore, based on the vascular nature, characteristic symptoms, pattern of the disease development, the absence of any bacterial, viral or nematodal signs and symptoms, or insect infestation, and the absence of a herbicide use in the past, I suspect the disease to be caused by a fungus.

4.6 Transmission (Possible)

Assuming it is a disease caused by a soil-inhabiting fungus such as Verticillium sp., there are following possible modes of transmission:

(i) seed-borne
(ii) vector (nematode)
(iii) mechanical

At this point, from the epidemiological data available, viz. the localized pattern of contiguous disease spread, seed transmission possibility can be just about eliminated. Were this disease seed-transmitted, the symptoms would appear at random in a population of affected trees. Similarly, since no insects were consistently associated with the diseased trees, insects too as vectors of the disease can be ruled out. Besides, if it were an insect-transmitted disease, it would be more uniformly and quickly spread, and have a more widespread incidence. Epidemiological data indicate a localized, contiguous spread of the disease at fairly a slow rate of spread. A soil transmission, through the agency of nematodes, is a distinct possibility based on the localized, contiguous pattern of the disease spread. Similarly, the appearance of the disease on new shoots arising at the base of a trunk cut close to the ground further suggests a connection of the disease with the soil. Finally, a mechanical transmission of the disease, through contaminated agricultural tools and implements, etc. is always a possibility in most infections, particularly in cases where no vectors for disease transmission are known.

5.0 RECOMMENDATIONS (TENTATIVE)

5.1 Technical/Scientific Recommendations:

The only technical/scientific recommendation is that this disease by studied further to characterize and confirm its etiology, mode of transmission, and to devise possible control measures.

5.2 Operational/Managerial:

In the absence of a confirmed laboratory diagnosis of the disease, few disease control recommendations or measures can be made at this time. However, some general agro-forestry preventive hygiene measures may be recommended:

1. Destroy the affected plants by burning.

2. Clean and sanitize all mechanical tools (shovels, axes, knives, etc.) that come in contact with diseased plants with hypochlorite solution (10%) before using these tools on healthy plants.

3. Do not plant new neem plantings without indexing seedlings for resistance to the disease. That would have to wait until the etiology is clearly established and confirmed, and a diagnostic test (e.g. ELISA) can be developed.

4. Observe closely further spread of the disease in Niger and surrounding areas, particularly in Nigeria and India, and impose a quarantine, if warranted. It is possible that the disease may have been imported from Indian subcontinent since neem is indigenous to the area, and is likely to have originated there due to availability and variability of the related germplasm (plant species) from Meliaceae.

5. Observe closely any nematodal infestations, incidental to or associated with the disease.

6.0 ACKNOWLEDGEMENTS

Although this report is presented by the author who takes full responsibility for any conclusions, opinions, omissions, and commissions, it is truly the result of a team effort. Chief members of this team were Barry Rands, without whose expertise, we would have technologically gotten nowhere, Hamadou Bourahima without whose knowledge about Niger, his introductions to government officials, and language and cultural interpretations, we would have gotten metaphorically nowhere, and his competent driver Hassan without whom we would have gotten literally nowhere.

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I thank Madame Corey Brooymous of DFPV at AGRHYMET in Niamey for allowing me to review work done on the project in her laboratory and to Mr. Hassane Hama of Phytopathology and Entomology laboratories at INRAN, Maradi, for allowing us to use their laboratory facilities, particularly on the weekend. My thanks are to Ambassador and Mrs. Carl Cundiff for inviting me July 4 celebration and wonderful American hamburgers at their home in Niamey. I also thank Dr. Wiley N. Garrett, Professor and Chairman, Dr. Charles Mims, Professor, Dr. Cedric Kuhn, Professor (retired), Dr. William Powell, Professor, and Dr. Ronald Roncadori, Professor, Division of Plant Pathology, College of Agriculture, at the University of Georgia, Athens, Georgia for providing facilities to complete portions of this investigation, and for their valuable input of helpful suggestions, discussions, critique, advice, and peer review. Needless to say that without the support of the wonderful gang at Biosystems/Atlanta, this assignment would not have been completed, on time and within budget, as usual.

7.0 REFERENCES

Anonymous (1990). Can A "Pesticide Tree" Such As Neem Have Serious Insect Pests? International Ag-Sieve Vol. 2(3), 32-4 to 32-5.

Hegde, N.G. (1990). Neem: Silviculture and Production. BAIF Development Research Foundation, Pune, India.

Kerkhof, P. (1990). Agro-forestry in Africa - A survey of project experience. Chapter 12. Majjia Valley Windbreak project, pp. 113-

123. The Panos Publications, Ltd., London, U.K. 216 p.

Ketkar, C.M. (1976). Utilization of Neem (Azadirakhta indica Juss. and Its Bye-Products). Report of the Modified Neem Cake Manurial Project, 1969-1976. Directorate of Non-edible Oils and Soap Industry, Khadi and Village Industries Commission, 3 Irla Road, Vile Parle (West), Bombay 400 056, India., 234 p.

Knausenberger, Walter I. (1991) Specimens of Neem Disorder Follow-up. A USAID-Niger/AFR/TR/ANR Memorandum dated January 31, 1991 to George Taylor, USAID/Niger/ADO. 2 p.

Lake Chad Basin Commission (1989). Control of cochineal infestation on neem trees among the member of states of the Lake Chad Basin Commission. Joint Project LCBC/OAU/IAPSC, N'Djamena, TCHAD, October 1989, 15 p.

Mitra, C.R. (1963). Neem. Indian Central Oil Seeds Committee, Hyderabad, India. March 1963. 186 p. + Appendix.

Ritchie, A. (1990). Initial assessment of the potential for the production and use of neem products in Niger. West Africa Regional Technical Advisory Team, Care International Au Niger, Niamey, Niger. 49 p. + 3 Appendices.

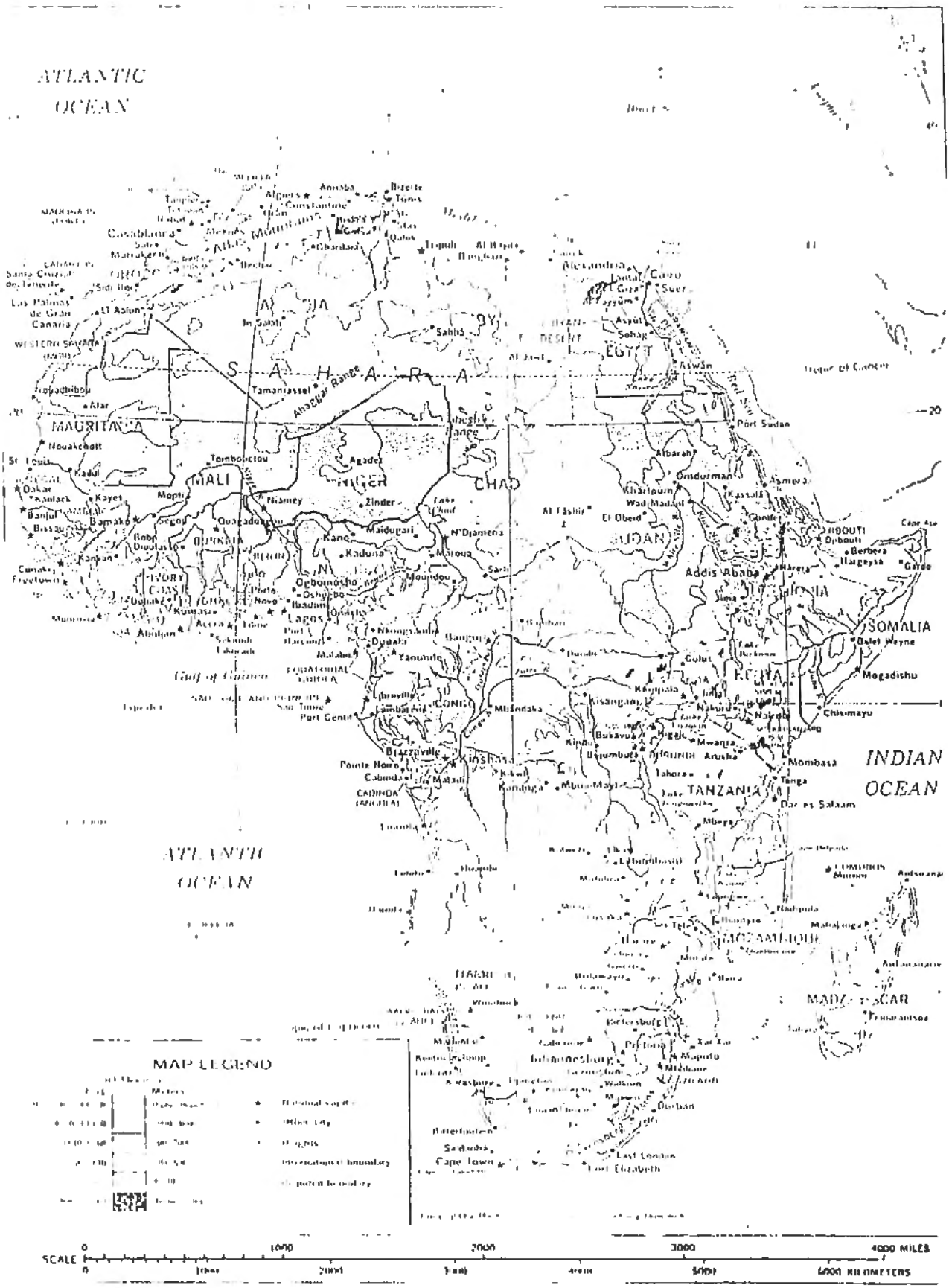
Shukla, P.C. and Desai, M.C. (1988). Neem as a source of cattle feed. The International Tree Crops Journal, No. 5.

Sinniah, D., Varghese, G, Baskaran, G., and Koo, S.H. (1983). The fungal flora of neem (Azadirachta indica) seeds and neem oil toxicity (Aspergillus, aflatoxin). Malays Appl. Bio., Kuala Lumper: Malaysian Society of Applied Biology, June 1983, v. 12 (1), p. 1-4.

Van der Burg, G.P.G.M. and K. Hassane (1990). The exploitation potential of neem in Niger. Care International Au Niger, Niamey. 158 p. + 13 Appendices.

TABLE 1.
Epidemiology of A Systemic Disease of Neem in Niger

Location and Plantation size	Age (Yr)	Incidence	Severity	Pattern of Spread	Stump Shoots Diseased
Mayahi Plantation 60 acres	7-9	40-50%	Moderate to high	Localized	Yes
Sani-Tanko (Gadabeji) 4 trees	5-7	100%	Moderate to high	General	Yes
USAID SMC Garangsou Est. 50 acres	5-7	75%+	High	Localized	No stump observed
Maggia Valley Near Tahoua >1.0 10_6 plants	12-15	10-20%	Low to Moderate	Localized	Yes
Tahoua Beltway Nursery 1800 seedlings	0.25	1.5%	Low to Moderate	Random	N/A
Groumdji Roadside Size unknown	6-7	25-50%	Low to Moderate	Localized	No stump observed
Birni 'N Konni Roadside One tree	10-15	Only tree	High	N/A	Yes
Dosso Roadside Size unknown	8-10	25-50%	Moderate to high	Localized	No stump observed
Niamey 1 Roadside Est. 50 trees	10-15	0%	N/A	N/A	No stump observed
Niamey 2 USE Rec Ctr Est. 20 treess	8-10	0%	N/A	N/A	No
Niamey 3 Roadside Est. 50 trees	6-8	40-50%	Low to Moderate	Localized	No stump observed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INDIAN OCEAN
ATLANTIC OCEAN
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MAURITANIA
MALI
NIGER
CHAD
SUDAN
EGYPT
LIBYA
ALGERIA
NIGERIA
ETHIOPIA
SOMALIA
KENYA
TANZANIA
MADAGASCAR
Gulf of Guinea
Tropic of Cancer
Equator
Cape Town
Johannesburg
Durban
Nairobi
Addis Ababa
Khartoum
Lagos
Kinshasa
Luanda
Dakar
Cairo
Algiers
Tripoli
Timbuktu
Agadez
Niamey
Kano
Maiduguri
N'Djamena
Mogadishu
Dar es Salaam
Mombasa
MAP LEGEND
SCALE
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4000 MILES
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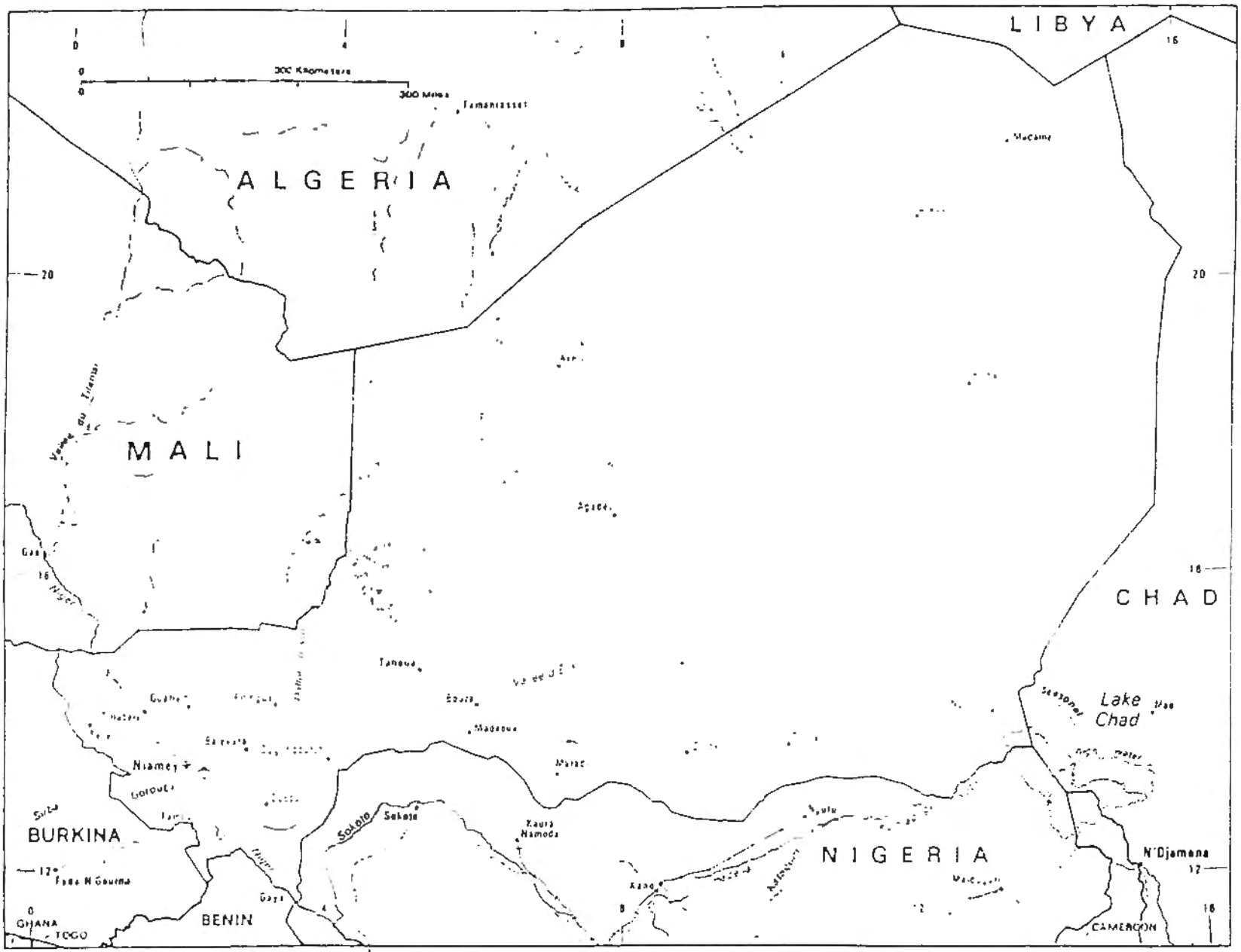
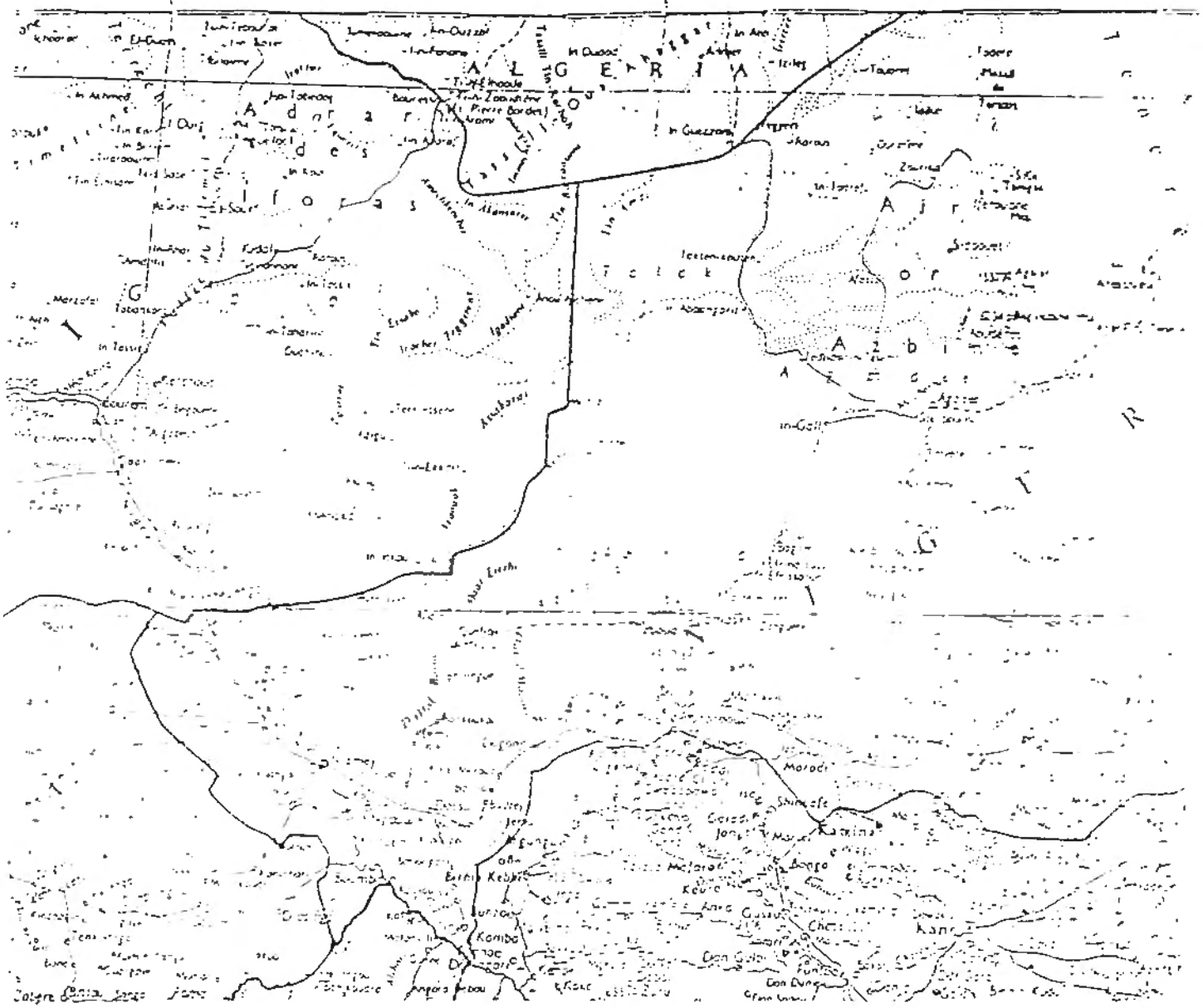
5000
6000 KILOMETERS

Niger


LIBYA
ALGERIA
MALI
CHAD
NIGERIA
BURKINA
BENIN
GHANA
TOGO
CAMEROON
Lake Chad
Niamey
N'Djamena
Tamanrasset
Agadez
Tahoua
Maradi
Zinder
Sokoto
Kaura Namoda
Gaya
Fada N'Gourma
300 Kilometers
300 Miles
20
16
12
8
4
0
International boundary
National capital
Railroad
Road
International airport
Boundary representation is not necessarily authoritative

Courtesy George F. Hill & Son, Ltd



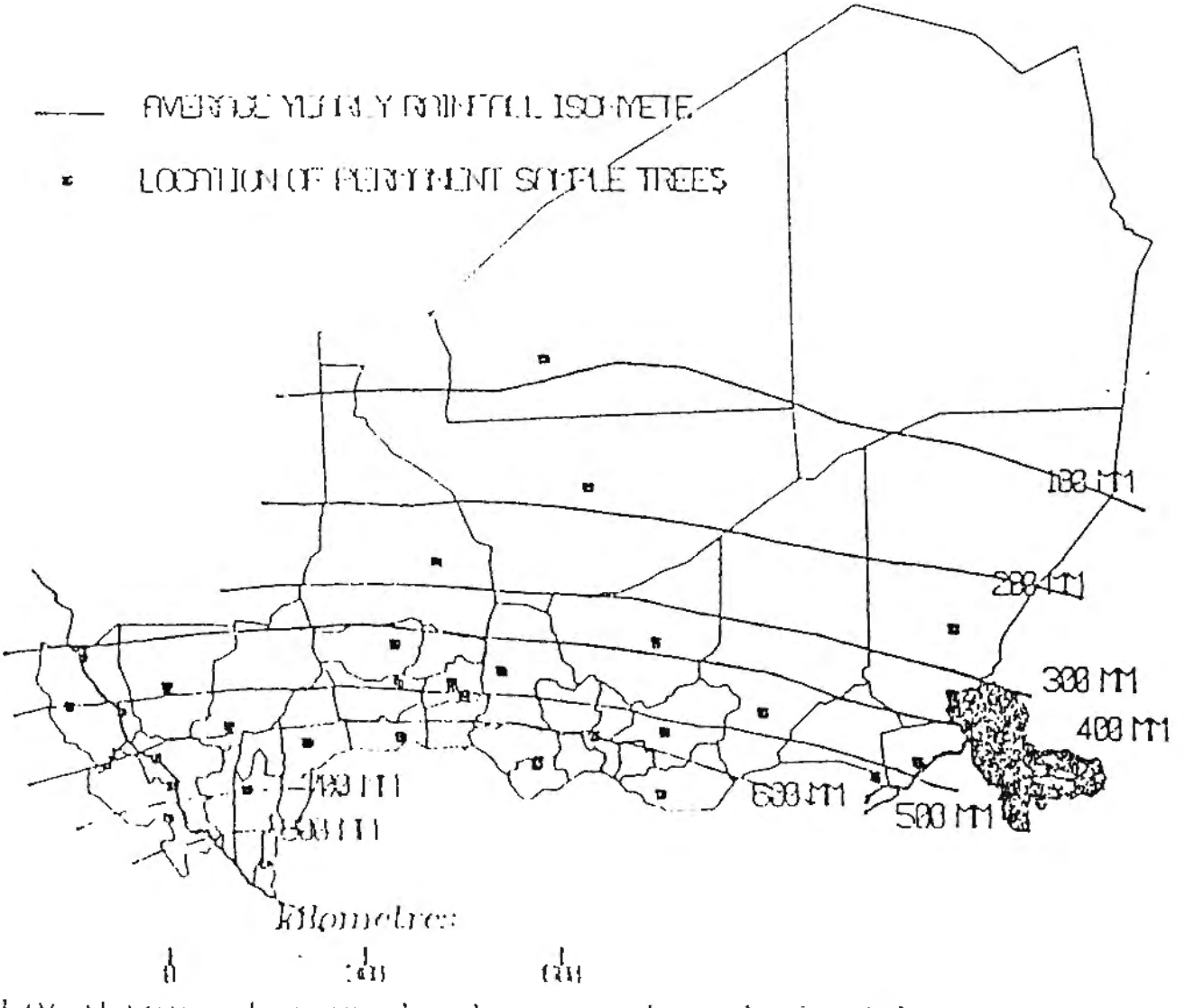
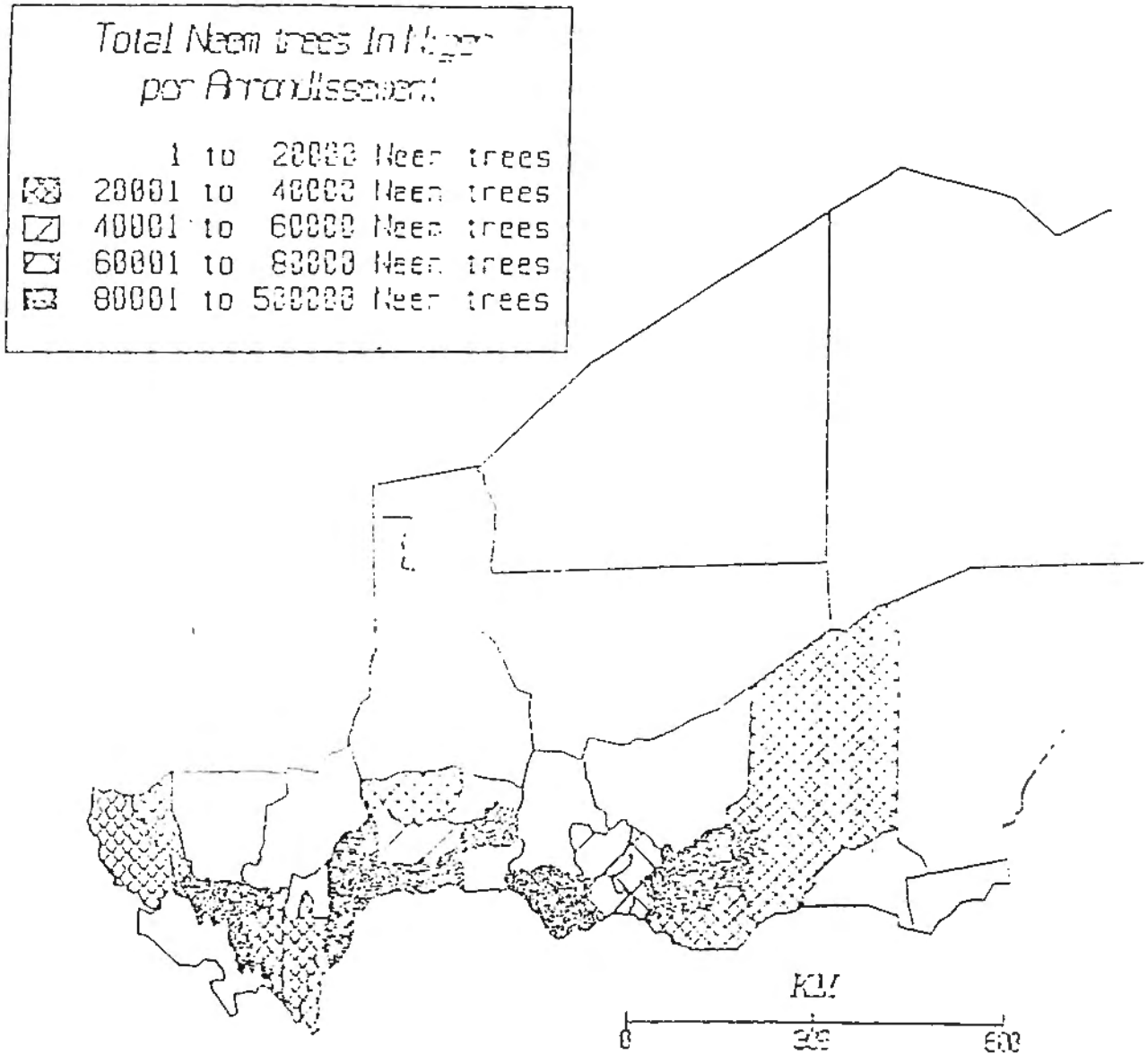


Figure 1: Location of sample tree network in Niger.

Neem trees located in villages.



Density of Neem trees in Niger.

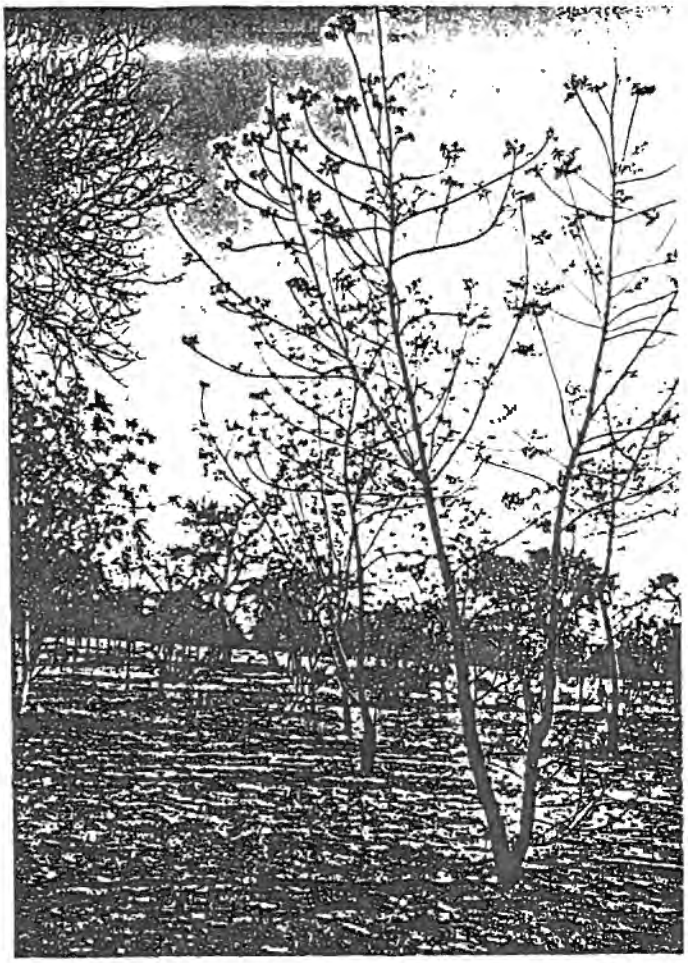


Fig. 1.
Mayahi Plantation devastated by the disease. Note spindle-top appearance of branches.

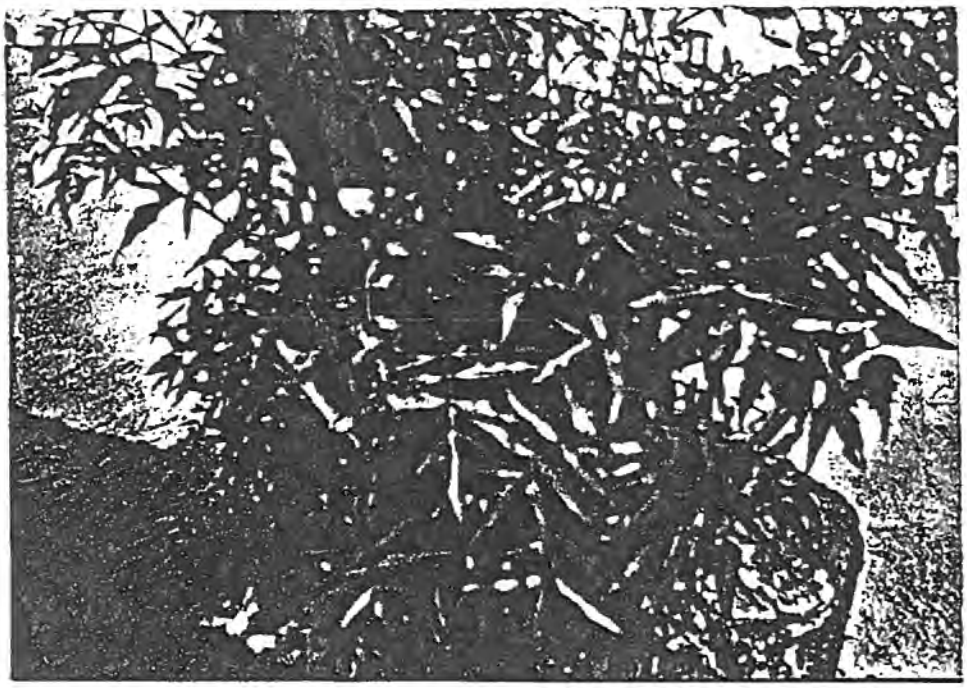


Fig. 2.
Off-shoots from the base of stump. Crinkly, yellow, deform leaves are jus below the hanc (out of focus)

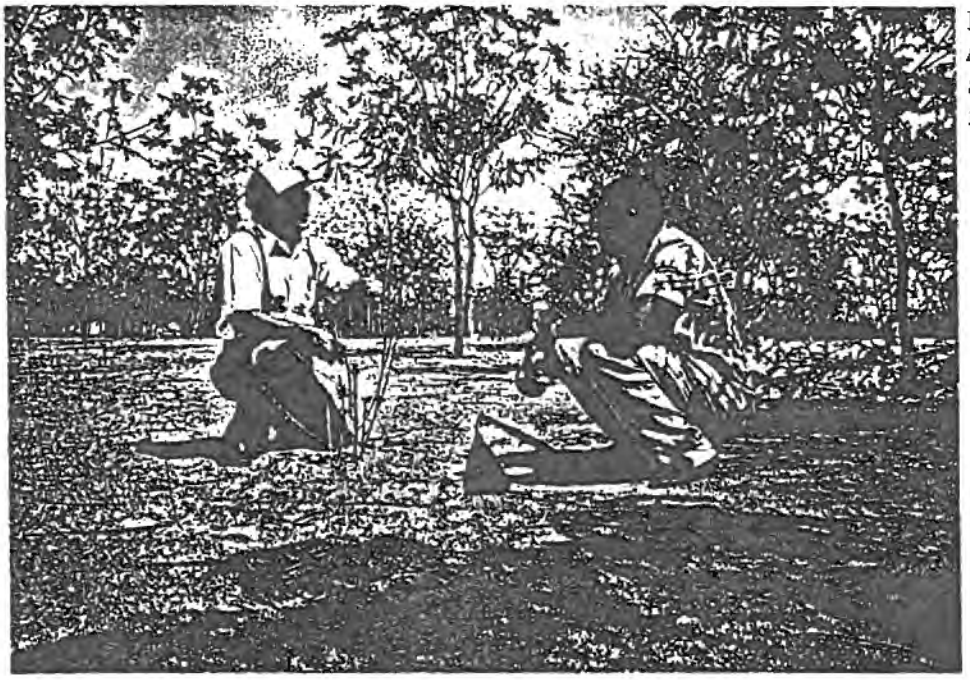


Fig. 3.
A dying neem tree
about to be dug up
for root examinati

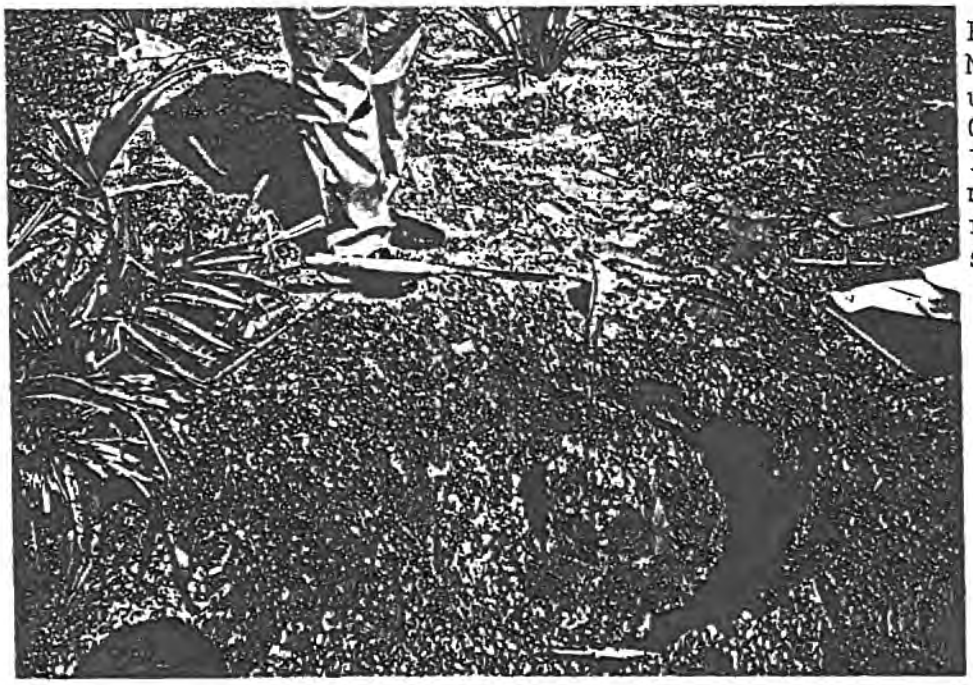


Fig. 4.
Method of digging
up dying neem tre
Central ball is
left intact so as
not to damage tap
root system. Note
sandy soil.

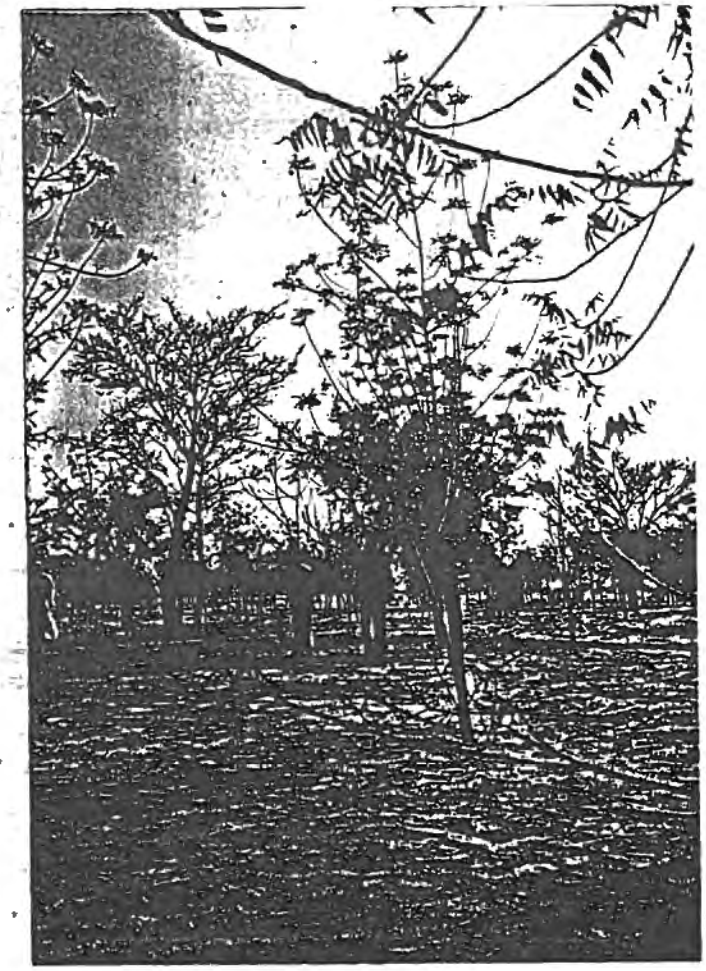


Fig. 5. A dying neem tree in the foreground. Several similarly affected trees are in the background as well.

Fig. 6. A dead neem tree in the foreground Note spindle-top branches as well as our camel (Toyota Landcruiser) in the Sahel in the background.

Fig. 7.
The farm in Sani-Tanko village near Gadabeji, Nige where disease was first noticed in 1990.

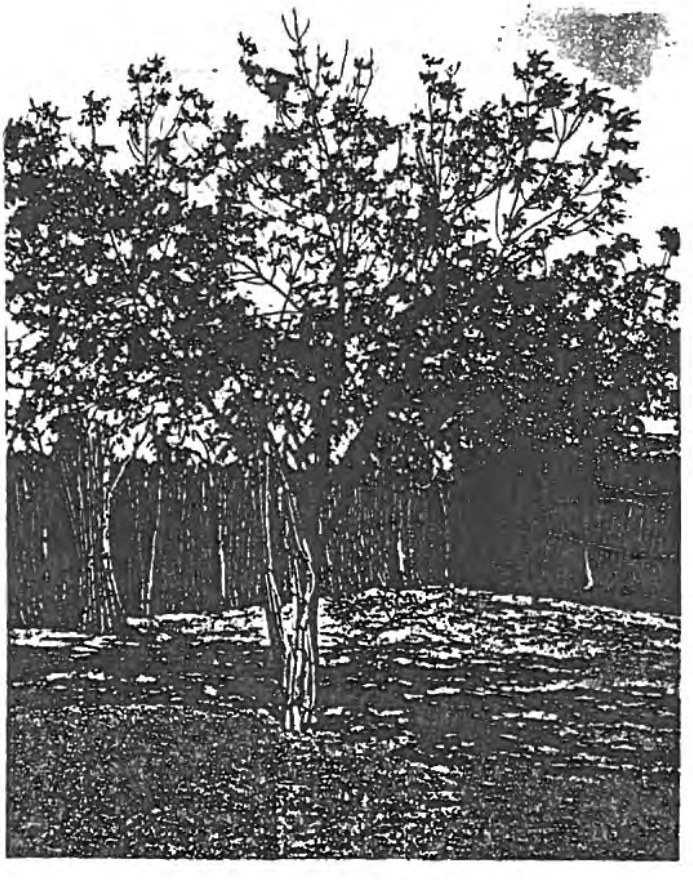


Fig. 8.
Five-year old dying neem tree with characteristic symptoms of disease, in the courtyard of the farmhouse above (Fig. 7.).

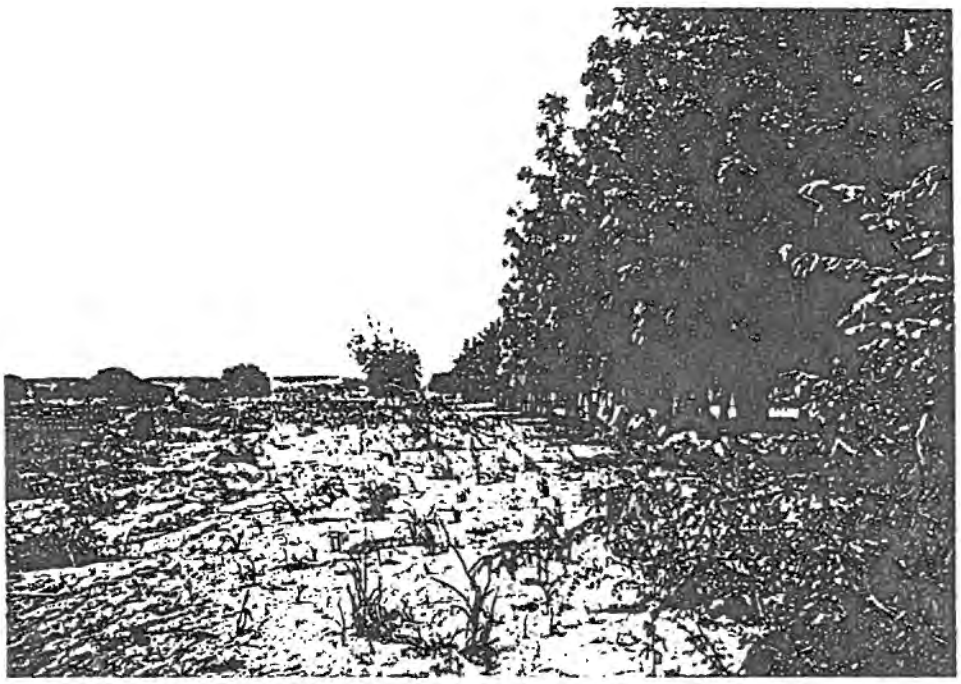


Fig. 9.
Maggia Valley
Wind Break
Project near
Bouza. Note a
row of healthy
neem trees on
right. An
Acacia plant is
in the right
foreground while
a poor crop of
millet can be se
in the middle of
photograph.

Fig. 10.
A fuelwood
gatherer in
Maggia Valley
Windbreak Projec
Three piles of
neemwood gathere
represents a day
work and "will
feed a family of
five.". she saic

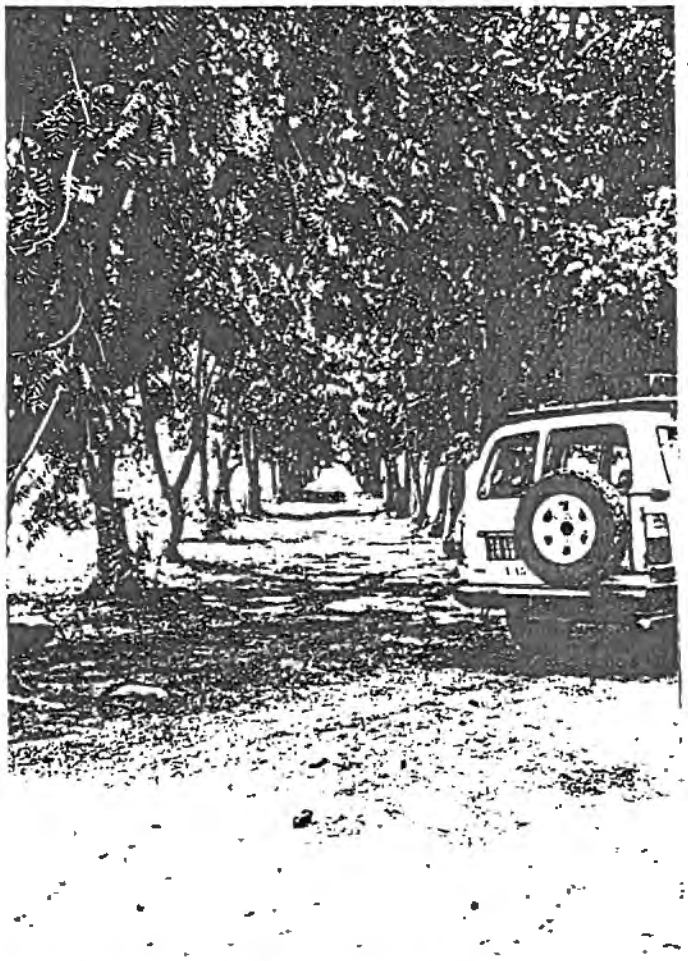


Fig. 11. Maggia Valley Windbreak Project. Note diseased tree in the left back-ground, and a stump with off-shoots behind the tree in the left foreground

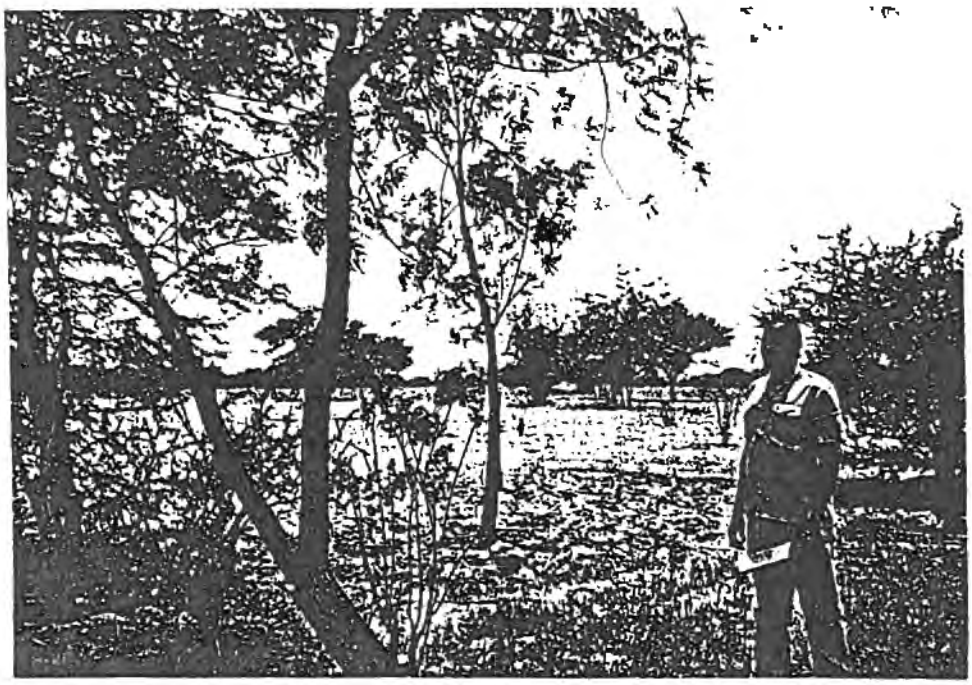


Fig. 12. Maggia Valley Wind Break Project. Note two dying trees in the left foreground, and several dying, dead, or missing trees in the center and background.

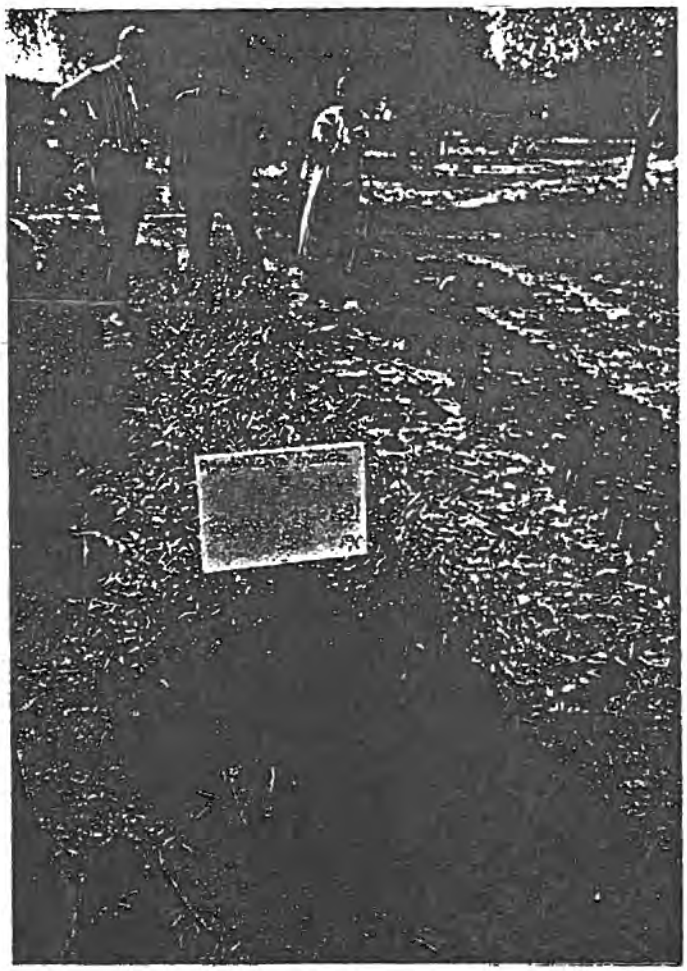


Fig. 13. Tahoua Beltway Nursery. Three-month old seedlings of neem showed 1.5% incidence of disease.

Fig. 14. A close-up of 12-week old neem plants in Tahoua Beltway Nursery showing characteristic disease symptoms.

Appendix A

BIOSYSTEMS/ATLANTA

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762 U.S. HIGHWAY 78 · LOGANVILLE, GEORGIA 30249 (404) 466-1511 FAX (404) 466-1512

LABORATORY ANALYSIS REPORT

August 14, 1991 Report No. 278-9206

Sponsor:
Mr. Bruce Crossan
USDA/OCID/DRD/NRE
3211 South Building
Washington, DC 20250-4300

Sample Description ..... Neem plants, seeds, soil
Sampling Date and Time ..... 06/28/1991 to 07/05/1991
Sampled by ..... Biosystems
Sample Delivered by ..... Biosystems
Sample Received in Lab ..... 07/16/1991 1030 Hrs
Container & Packaging ..... Plastic bags
Sample Temperature on Receipt ..... Ambient
Sample Processed 07/16/1991
Results Reported by Telephone/Fax ..... No

Sample No.	Sponsor ID	Test	Results
9206-1 soil	Soil Maggia Valley	Nematodes	
		Rootknot	0/100 g
		Sting	0/100 g
		Lance	0/100 g
		Reniform	0/100 g
		Lesion	0/100 g
		Stubby-Root	0/100 g
		Ring	0/100 g
		Stunt	0/100 g
		Spiral	0/100 g
		Dagger	0/100 g
		Sheath	0/100 g
		Cyst, Females	0/100 g
		Cyst, Larvae	0/100 g
		Cystoid	0/100 g
9206-2 soil	Soil USAID Seedling Replication Center	Nematodes	
		Rootknot	0/100 g
		Sting	0/100 g
		Lance	0/100 g
		Reniform	0/100 g
		Lesion	0/100 g
		Stubby-Root	0/100 g
		Ring	28/100 g

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Sample Processed 07/16/1991
Results Reported by Telephone/Fax ..... No

Sample No.	Sponsor ID	Test	Results
9206-1 soil	Soil Maggia Valley	Nematodes	
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		Lance	0/100 g
		Reniform	0/100 g
		Lesion	0/100 g
		Stubby-Root	0/100 g
		Ring	0/100 g
		Stunt	0/100 g
		Spiral	0/100 g
		Dagger	0/100 g
		Sheath	0/100 g
		Cyst, Females	0/100 g
		Cyst, Larvae	0/100 g
		Cystoid	0/100 g
9206-2 soil	Soil USAID Seedling Replication Center	Nematodes	
		Rootknot	0/100 g
		Sting	0/100 g
		Lance	0/100 g
		Reniform	0/100 g
		Lesion	0/100 g
		Stubby-Root	0/100 g
		Ring	28/100 g

		Stunt	0/100 g
		Spiral	0/100 g
		Dagger	0/100 g
		Sheath	0/100 g
		Cyst, Females	0/100 g
		Cyst, Larvae	0/100 g
		Cystoid	0/100 g
9206-3 soil	Soil Tahoua Beltway Nursery (with presumed healthy neem seedling)	Nematodes	
		Rootknot	0/100 g
		Sting	0/100 g
		Lance	0/100 g
		Reniform	0/100 g
		Lesion	0/100 g
		Stubby-Root	0/100 g
		Ring	0/100 g
		Stunt	0/100 g
		Spiral	0/100 g
		Dagger	0/100 g
		Sheath	0/100 g
		Cyst, Females	0/100 g
		Cyst, Larvae	0/100 g
		Cystoid	0/100 g
9206-4 soil	Soil Tahoua Beltway Nursery (with presumed diseased neem seedling)	Nematodes	
		Rootknot	0/100 g
		Sting	0/100 g
		Lance	0/100 g
		Reniform	0/100 g
		Lesion	0/100 g
		Stubby-Root	0/100 g
		Ring	0/100 g
		Stunt	0/100 g
		Spiral	0/100 g
		Dagger	0/100 g
		Sheath	0/100 g
		Cyst, Females	0/100 g
		Cyst, Larvae	0/100 g
		Cystoid	0/100 g

LABORATORY ANALYSIS REPORT

9206-5 seeds	Neem seeds Maggia Valley	Standard plate count Yeasts and molds	8.7x10E+04 cfu/g 2.6x10E+04 cfu/g
9206-6 leaves	Neem leaves (Healthy) Mayahi	Standard plate count Yeasts and molds	118 cfu/g 63 cfu/g
9206-7 leaves	Neem leaves (Diseased) Birni 'O Konni	Standard plate count Yeasts and molds	58 cfu/g 87 cfu/g
9206-8 leaves	Neem leaves (Healthy) Maggia Valley	Standard plate count Yeasts and molds	107 cfu/g 93 cfu/g
9206-9 leaves	Neem leaves (Healthy) USAID Seedling Replication Center	Standard plate count Yeasts and molds	132 cfu/g 100 cfu/g
9206-10 leaves	Neem leaves (Diseased) Maggia Valley	Standard plate count Yeasts and molds	203 cfu/g 176 cfu/g
9206-11 leaves	Neem leaves (Diseased) Mayahi	Standard plate count Yeasts and molds	140 cfu/g 111 cfu/g
9206-12 leaves	Neem leaves (Diseased) USAID Seedling Replication Center	Standard plate count Yeasts and molds	235 cfu/g 193 cfu/g

LABORATORY ANALYSIS REPORT

9206-3A leaves	Seedling (presumed diseased) Tahoua Beltway Nursery	Fungi (EM)	Detected (see electron micrographs 54314 - 54319)
		Viruses (EM)	None detected (see electron micrograph 54320)
9206-4A stem	Seedling (presumed diseased) Tahoua Beltway Nursery	Fungi (EM)	None detected
		Viruses (EM)	None detected (see electron micrographs 54321 -54323)

==========

Abbreviations and Notations:

cfu = colony forming units
cm = centimeter
E = Exponentiation e.g. 6.02E+05 means 602,000
l = liter
mg = milligram
ml = milliliter
NA = Not available/not applicable
ug = microrgram
umhos = micro mhos/cm (specific conductance)

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BIOSYSTEMS, INC.

G.K. Batra, Ph.D.
Technical Director

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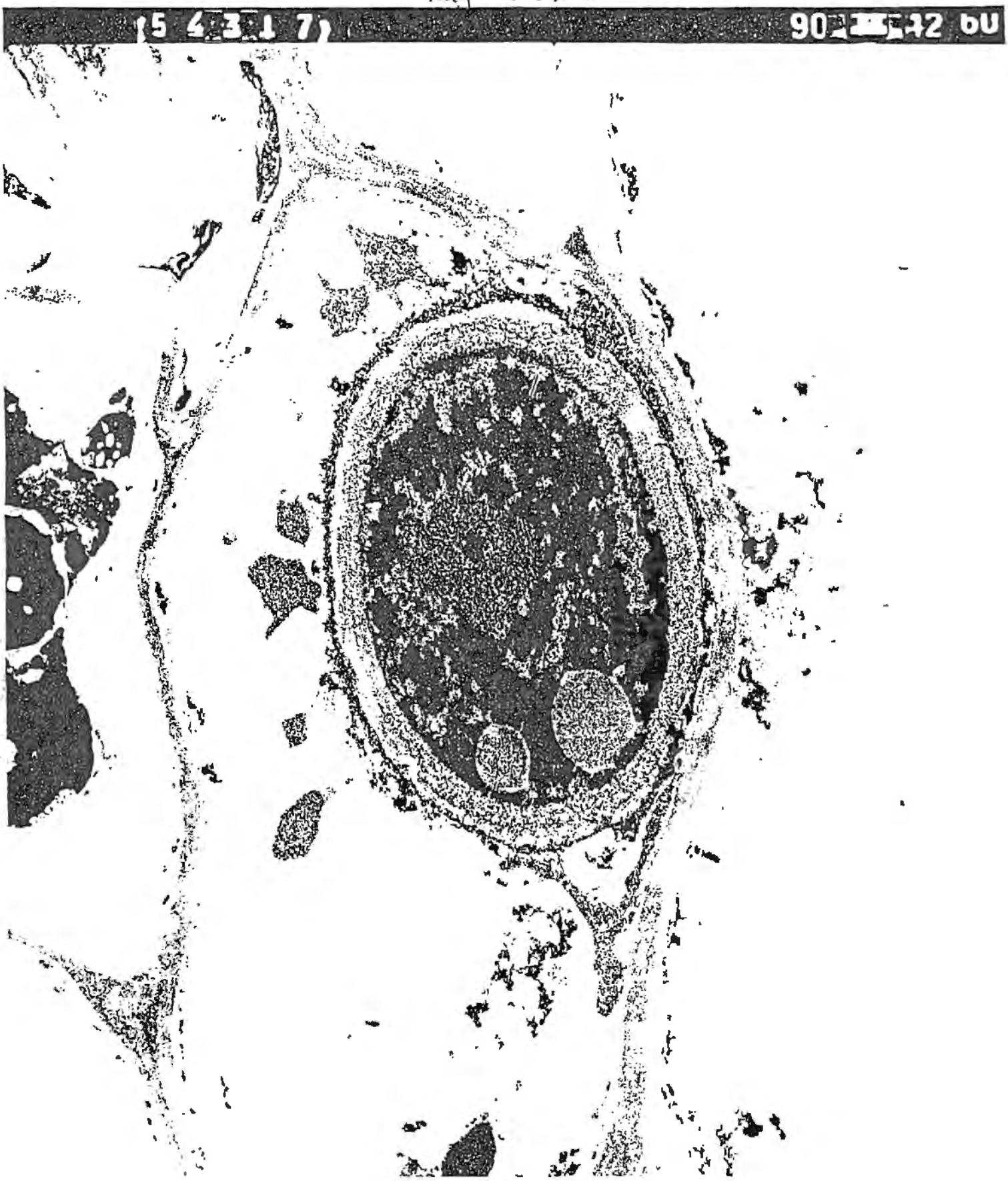
Mag. 12.5×10^3

2

mag. 10.0×10^3



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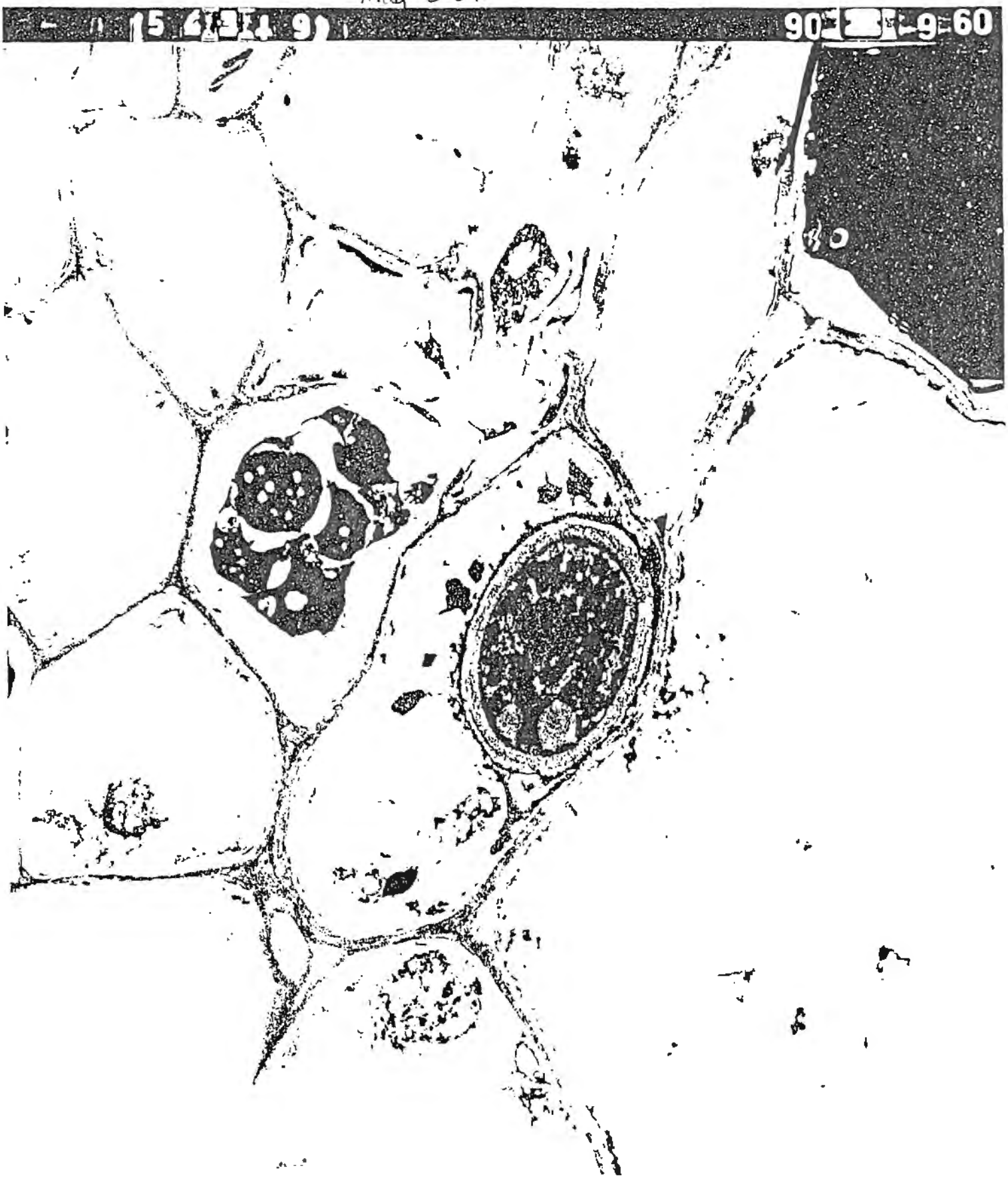
(5 4 3 1 8) 90 14 60

Mag 5.0 X10^3



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Mag. 80.0×10^3

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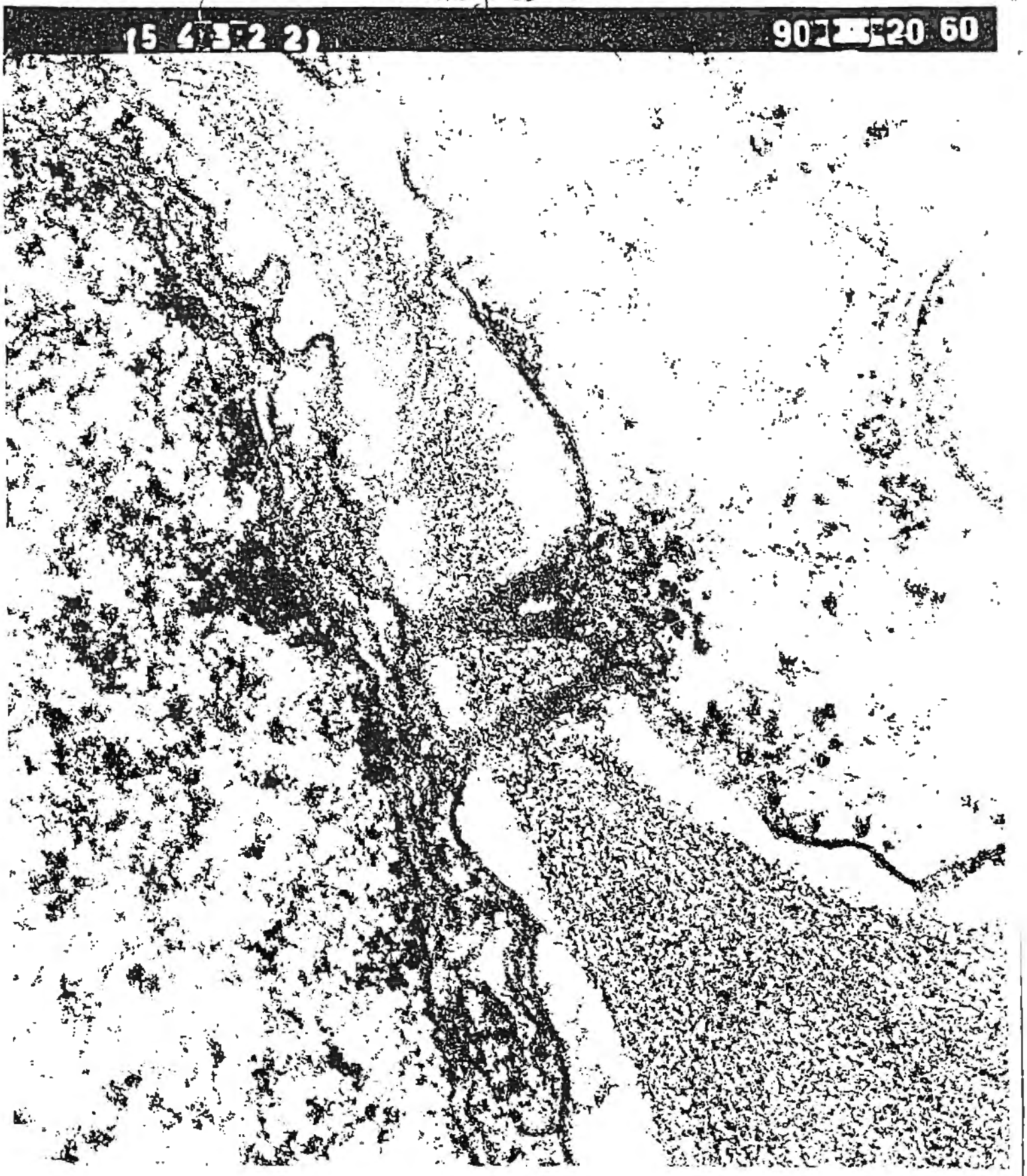

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